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PHYSIOLOGICAL REVIEWS

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HISTAMINE

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The isolation of β-iminazolylethylamine from ergot and the study of its effects upon animals were the starting points of investigations, the branches of which have now touched numerous aspects of the biological sciences. The fact that the pharmacodynamically active amine may be formed by decarboxylation of the amino-acid histidine, suggested the appropriate name *histamine*, which, after the abbreviation β-I. had been used for a few years, gained general acceptance.

The early studies of the action of histamine were strictly pharmacological since there was then no evidence establishing the presence of the amine in normal tissues or suggesting its liberation from them under physiological conditions. The similarity between the effects of large doses of histamine, the signs of anaphylactic shock in animals, and those of secondary wound shock in man led to the supposition that histamine or a closely related substance might be formed under pathological conditions. The production of histamine from histidine by members of the colon group of micro-organisms, which probably takes place in the intestine as well as *in vitro*, has been extensively studied. The results of the recent investigations of the reactions of the vessels of the human skin, in which histamine has played an important part, have illuminated many points which are of great importance in physiology, general medicine, pathology and immunology. A sound physiological foundation to support the future development of dermatology is to be found in the results of these studies. Histamine is now used in medicine as a stimulant of gastric secretion and in certain tests for the efficiency of the circulation. In physiological investigations it has been very extensively used to stimulate smooth muscle or to lower blood pressure. It is apparent, therefore, that interest in histamine is shared by a great many groups of biological workers.

Although there is as yet no satisfactory evidence that the amount of histamine in a tissue is changed under physiological conditions, the

demonstration of the amine in perfectly fresh normal tissues has materially enhanced the possibility that it plays a physiological rôle. It should be possible to gain further information on this important point in the near future. The time is opportune for taking stock of the knowledge of the properties of this amine. Quite recently a review of certain aspects of this subject has appeared in Ergebnisse der Physiologie (Küpper, 1930). The Monograph on *Histamine* by Feldberg and Schilf (1930) provides a comprehensive review of published data and in addition contains a great deal of information secured by these investigators which has not yet been published in detail. Certain important aspects of the subject have been recently discussed by Dale (1929). Although the mass of data is in some instances confusing, we have attempted to collect it here in the hope that future reviewers who interest themselves in the subject will be spared a certain amount of labour. Short résumés have been inserted at the end of certain sections of this review. Usually we have considered it unnecessary to summarize the shorter divisions, and in many cases the character of the information available does not justify an attempt to summarize the longer sections.

ANALYTICAL METHODS. Methods for the estimation of histamine may be divided into two general groups, chemical and physiological. Both groups are used for qualitative and quantitative determinations. Among the chemical methods may be included the qualitative colorimetric tests, the development of these reactions for quantitative work, and the procedures for the isolation and absolute identification of histamine. The physiological effects produced by histamine have been used to demonstrate its presence in extracts of tissues, and have been extensively used for quantitative work. The various methods may be tabulated as follows:

	Qualitative	*Quantitative*
Chemical	Pauly reaction Knoop reaction Zimmerman reaction Isolation as picrate or as other salts	Koessler and Hanke modification of the Pauly reaction
Physiological	Contraction of isolated guinea pig-uterus or intestine. Lowering of blood pressure in cats or dogs Production of wheals in the skin of human subjects Bronchospasm in guinea-pigs. Effect on isolated uteri of mice or rats	All these qualitative methods, with the exception of those involving effects on the uterus of the mouse or rat, have been utilized for quantitative determinations

It should be noted that certain precautions must be taken in handling any tissue the normal histamine content of which is being studied. The tissue must be fresh. Precautions to avoid bacterial production or destruction of histamine, and also to prevent the possibility of the loss of the amine through the action of histaminase, should be taken. The rate of change of the histamine content of tissue after removal from the body has not yet received adequate study, but it appears unlikely that the changes are as rapid as has been supposed. The avoidance of bacterial action must be considered at every step in the procedures used in fractionating solutions which are thought to contain histamine. Histamine, even in pure solution, rapidly loses its potency at room temperature if precautions to prevent growth of bacteria are not taken. Sterile histamine solutions retain their potency for long periods at room or icebox temperatures.

For estimation the histamine in a tissue must be brought into solution and this may be accomplished by extraction with water, saline, dilute alcohol, or by complete disintegration of the tissue with boiling hydrochloric acid. Many extraction procedures do not give a complete yield of the histamine present. Work in our laboratory has shown that the first extraction with a moderate amount of alcohol may remove approximately 60 per cent of the total histamine, the second 30 per cent, the third 5 per cent, while a fourth still leaves a small residue. The hydrochloric acid hydrolysis suggested by Hanke and Koessler (1920) is most useful. This brings all the histamine into solution, and the boiling treatment neither destroys the amine, nor produces it in detectable amounts from histidine, as was suggested by Abel and Kubota (1919).

For the physiological tests relatively little purification of the initial solution is required. Any of the chemical procedures involving precipitations or extractions result in loss of the amine, so that the final solution often contains only a fraction of the amount which was originally present.

In 1908 Kehrer described the use of the isolated uterus for the assay of ergot preparations and in 1910 Barger and Dale, after demonstrating that histamine was a constituent of ergot, showed it would cause the isolated guinea-pig uterus to contract. The technique for the use of the isolated uterus was thoroughly investigated by Dale and Laidlaw (1912) in their study of the methods of standardizing pituitary extracts and the same procedure has been employed for the physiological assay of histamine. This action of histamine is similar to that of "pituitrin," and

Abel suggested the use of histamine as a standard in this assay of "pituitrin." Guggenheim and Löffler (1916) advocated the use of isolated guinea-pig intestine instead of uterus. Strips cut from the intestine at any level from the duodenum to the cecum may be used. The whole organ may be kept cold and strips used as needed. Guinea-pig intestine was found to be more sensitive, but less regular in its action than rabbit intestine. This method has not been extensively employed in recent years, but has been adopted by Kendall (1927) in his study of the inactivation of histamine by formaldehyde.

By far the most useful method for the qualitative and quantitative physiological determination of histamine is that based on the lowering of the blood pressure of an etherized and atropinized cat. This method has been developed and extensively used in Dale's laboratory. The technique is fully described by Burn (1928).

Histamine produces characteristic wheals and flares in the skin of human subjects and in that of certain laboratory animals, when administered intracutaneously. This reaction has not been extensively employed, but extracts are frequently said to contain histamine when they produce the skin reaction as well as the other biological responses. Harris (1927) has employed the skin reaction to obtain approximate estimates of the amount of histamine-like material in a solution.

Koessler, Lewis and Walker (1927) developed a technique by which histamine and other substances could be assayed by the production of bronchospasms in guinea-pigs. Large guinea-pigs were prepared for artificial respiration and records of the intrathoracic pressure were secured. Injections of the solution to be tested were made into the external jugular vein. Amounts of histamine as small as 0.005 mgm. could be detected. In the published papers no results of quantitative assays are given, but from a private communication and from our limited use of the procedure we believe that the method yields fairly satisfactory results.

In addition to the isolation and absolute identification of histamine, two colorimetric methods have been devised for its estimation, and one of them has been employed extensively. This method depends on the production of coloured compounds resulting from linkage between the iminazole ring and diazo substances. These diazo compounds were discovered by Griess in 1866 and their formation first utilized for diagnostic purposes by Ehrlich in 1882. Ehrlich developed a test which he stated was valuable in the diagnosis of typhoid fever and other conditions. After the addition of the diazo reagent to urine a primary colour develops, due probably to bilirubin, and a secondary colour follows the

addition of excess ammonia. This secondary colour Ehrlich believed to be due to phenols. In Ehrlich's hands the test was not employed for the identification of any particular compound or class of compounds.

The diazo reaction was shown by Pauly (1905) to be positive for histidine, and his name is often associated with the test when it is used for the detection of iminazoles. For the test a fresh solution of diazobenzenesulphonic acid is prepared by adding sodium nitrite to a solution of sulphanilic acid in dilute hydrochloric acid. The solution to be tested is made strongly alkaline with sodium carbonate and the diazobenzenesulphonic acid is added. A positive reaction shows, within three minutes, as a dark cherry red colour, which persists on dilution and changes to orange when the solution is made acid. Histidine responds to this test in concentration as low as 1:100,000. Of the various protein-split products investigated only tyrosine and histidine give the above reaction. The tyrosine solution becomes yellow on dilution and a bronze yellow on acidification. The possibility of tyrosine being present can be excluded if the solution gives a negative Millon reaction. The diazo reaction was found by Pauly to be positive for histidine whether free or bound in a protein.

In a footnote to one of his papers, Pauly stated that histidine gave the diazo reaction because it contained the iminazole nucleus, but he also said that pyrrol derivatives and nitrogen compounds with a ring configuration somewhat similar to the iminazole grouping might also give the diazo test. This was misunderstood by Burian who suggested that the reaction could be used to distinguish between iminazole and pyrimidine compounds. Pauly later showed that as a general rule this could not be done. Pauly made no attempt to apply the diazo reaction to iminazole compounds other than histidine, nor did he use it for quantitative work.

The first application of the Pauly reaction to quantitative estimations (again only for histidine) was made by Weiss and Ssobolew in 1914. These authors found that under certain definite conditions a maximum colour was developed in the reaction when 1 mgm. of histidine dichloride was present. Less intense colours were produced when smaller or larger amounts of histidine were present.

In a series of papers published between 1919 and 1924 Koessler and Hanke described an improved technique for carrying out the diazo reaction, methods for its use in quantitative work, and for the preparation of purified solutions to which the colorimetric method of assay could be applied. Koessler and Hanke extended the method to include the esti-

mation of a number of iminazoles, and found that these could be estimated in mixtures or in pure solutions. They state that 0.00001 gram of any of the iminazoles can be estimated with considerable accuracy.

The purification procedures necessary for the colorimetric estimation of histamine are complicated and a loss of the amine is not only possible but probable at almost every stage. The final amount secured may not indicate accurately the amount initially present in the tissue. However, purification of the solution is essential if the diazo reaction is to be applied. The diazo reaction is not specific for histamine, nor indeed for iminazoles. Any histidine present must of course be removed and also phenols, but there are other interfering substances, unknown in composition, which may mask the colour reaction.

To produce the colour Koessler and Hanke, like other workers, used p-diazobenzenesulphonic acid. This is prepared by adding sodium nitrite to a hydrochloric acid solution of sulphanilic acid. In preparing the reagent a number of precautions must be observed. The colour is matched against a standard solution made by mixing definite quantities of solutions of methyl orange and congo red. Koessler and Hanke have prepared tables for the calculation of the results. They claimed that amounts of histamine as small as 0.1 mgm. could be determined accurately, and also that they were able to recover 99 per cent of histamine added to casein or blood and other tissues. In the hands of other workers the method has not been so successful.

Several criticisms of the Koessler-Hanke procedures have been advanced: 1. That a great deal of histamine is lost in the various precipitations. Gerard (1922), for example, found that histamine was adsorbed by the humin material formed during the acid hydrolysis of the tissue. Thorpe (1928) and others have shown that as much as 5 per cent of histamine may be lost in the phosphotungstate precipitation. 2. Standard colour solutions may not always be equivalent, due to impurities in the dyes. Hunter (1925) found that Grübler methyl orange and congo red were by no means pure and that the amount of impurity varied. Koessler and Hanke's tables were based on the matching of "standard" indicator solutions and obviously the accuracy would be lessened if the indicators were different. To overcome this, Hunter suggested not only purification of the dyes before use but also the evaluation of the indicators in terms of pure histamine for each lot of dye. Such a procedure is laborious but it increases the degree of accuracy of the method. 3. Even after the lengthy purification is performed the final solution will not always give a colour which matches the indicator in shade.

Lucas (1926) in a series of 13 extracts of liver, found that only in one case was a good match with the standard indicator secured. We have experienced a similar difficulty in using the method.

Although these difficulties are encountered the Koessler-Hanke method is the only chemical procedure thus far developed for the quantitative determination of histamine, and in many cases it is very useful.

Inouye (1913) observed that the diazo reaction could be applied more specifically to histidine after benzoylation. If the Schotten-Baumann reaction is applied to histidine by treating it with benzoyl chloride and sodium carbonate, histidine then forms a monobenzoyl derivative which still couples with the diazobenzenesulphonate. Tyrosine forms a dibenzoyl derivative which does not link with the diazo reagent and histamine has the iminazole ring ruptured, a tribenzoyl derivative being formed which does not give the diazo reaction. Obviously this is of no value for the determination of histamine but it does serve to distinguish it from histidine.

Histamine gives the colour reaction which Knoop (1908) discovered for histidine—a brownish red colour with bromine. As originally described by Knoop, the test is carried out by slightly acidifying the solution, adding bromine water in excess and then boiling off the excess bromine. Hunter (1922) proposed a modification of the Knoop method, particularly for use on deeply coloured solutions. To the solution to be tested bromine is added in excess and the mixture is extracted with chloroform until the chloroform layer is colourless. The aqueous residue is heated in a water bath and, if histidine or histamine is present, a red colour develops. Chloroform removes interfering pigments and excess bromine, without extracting the histidine or histamine-bromine complex. Knoop's reaction has not been adapted for quantitative work.

Zimmerman (1929) has recently discussed a colour reaction which is said to be specific for histamine. The reaction is carried out anaerobically in a Thunberg tube. In the tube is placed the solution to be tested, a few drops of cobalt nitrate solution are added, and in a small separate vial within the Thunberg tube some sodium hydroxide solution. The tubes are then evacuated and the sodium hydroxide mixed with the other liquid. If histamine is present a violet colour is produced. Zimmerman tested a number of substances, including histidine, and in each case the test was negative. Unfortunately this is not a sensitive reaction since quantities of histamine less than 0.5 mgm. will not respond.

In 1923 Whitehorn proposed a general method for the separation of basic substances such as ammonia and amines from solutions. These

were to be taken out on "permutit" and then removed from the "permutit" with a strong solution of potassium chloride. While Whitehorn showed that the method could be applied to histamine, no published account of such use has come to our notice. Work in our laboratory has shown that under certain circumstances this base-exchange method may be useful.

Itallie and Steenhauer (1925) have carefully described a series of distinctions between histamine and tyramine. These two bases are easily distinguished by the crystalline forms of such insoluble salts as the picrate, chloroplatinate, iodoplatinate, iodobismuthate, phosphotungstate and silicotungstate.

For the absolute identification of histamine it is essential that the base be isolated in relatively pure crystalline form and the properties of the crystals studied. In only a few cases has this been done. There is now no doubt that histamine is a constituent of ergot, of beef and horse lung and liver, of beef heart, and of beef muscle. In all these cases a long and tedious procedure has been carried out, resulting in the separation of crystals of histamine picrate. This is the only way at present known of establishing with certainty the presence of histamine in tissue. The procedure has been completely described by Best, Dale, Dudley and Thorpe (1927). Throughout the process heavy losses are encountered, but the losses are not sufficient at any stage to suggest the presence of any unidentified substance with physiological properties similar to those of histamine. This, and similar work, has clearly established the presence of physiologically large amounts of histamine in normal tissue.

To summarize the available methods of studying the histamine content of a tissue: Preliminary indications of the presence of the base may be secured by studying the effect of extracts on the blood pressure of an etherized cat, on the isolated guinea-pig uterus, or by production of characteristic bronchospasms in guinea-pigs. Similar but less definite information may be secured from the qualitative diazo or the Knoop reaction. The presence of histamine can be definitely established only by the isolation of the base in crystalline form. The inference that histamine is present is legitimate if the results of a complete physiological analysis of a solution are identical to those obtained with pure histamine. Once it is established that histamine is present in a certain tissue, quantitative measurements can often be made on suitably prepared extracts by the Koessler-Hanke procedure, or more simply and more accurately by the blood pressure method. A more satisfactory chemical method would be of considerable assistance.

DISTRIBUTION. The first isolations of histamine from biological sources were performed before any knowledge of its physiological activity had been gained. In 1909 Yoshimura obtained a base from the Japanese beverage, Tamari-shoyn, which is prepared from soy beans. This base had a composition represented by the formula $C_6H_9N_3$, and he surmised that it was derived from histidine. The identification of the base was not completed.

In their study of ergot, Barger and Dale (1910) isolated histamine and identified it chemically. In the same year Kutscher (1910) secured a base from ergot which resembled histamine chemically, but he considered it to have different physiological properties. These apparent distinctions have now disappeared and Kutscher's material was undoubtedly histamine.

Following the isolation from ergot, Dale and Laidlaw (1910) began the study of the physiological properties of histamine, and with the recognition of its pharmacodynamic effects and its high toxicity came deepened interest in its presence in animal tissue. A series of hypotheses regarding possible functions of histamine gradually developed and these affected the subsequent work. Ackermann (1910) showed that bacteria could produce histamine from histidine. Barger and Dale (1911) found it in intestinal mucosa, Mellanby and Twort (1912) demonstrated the presence of histamine-producing micro-organisms in the human intestine. These observations led to the hypothesis that histamine plays a rôle in the etiology of intestinal obstruction. The fact that histamine produces symptoms similar to those of anaphylactic shock led to the controversy over the presence of histamine in Witte's peptone and in pure proteins. The presence or absence of histamine was often decided on insufficient chemical or physiological evidence. In the period 1911–1927, with the possible exception of Abel's work, there was no convincing identification of histamine as a constituent of perfectly fresh normal tissue.

The question has frequently been raised whether histamine exists, as such, in any tissue. Can such a potent substance be present in the active state in living tissue? It has been postulated that it exists in loose combination with another substance, or that it exists in the cells from which it is liberated by physical or chemical means. Certainly the simplest extraction of fresh tissue yields histamine, and there is as much reason for considering it a constituent of normal tissue as for so considering insulin, epinephrine or any of the other hormones. On the other hand it is perfectly obvious that the hormones and histamine are very

loosely held in the tissues in which they occur, by some mechanism which normally prevents them from being liberated at a rate which is comparable to that resulting from their administration.

There is a limited number of cases in which histamine has been isolated as a chemical entity from a simple extract of fresh tissue, and in which precaution to avoid bacterial changes has been taken. In a number of cases the histamine was identified only by the diazo reaction, which is not a specific test for the amine. If the complete fractionation procedure devised by Koessler and Hanke (1919) has been carefully carried out prior to the addition of the recommended reagents, the identification of histamine by the colour reaction may be satisfactory. In many instances the presence of histamine has been inferred from physiological evidence, i.e., an extract produces many of the same effects as a solution of pure histamine. The lack of completeness of such evidence has led so careful a worker as Sir Thomas Lewis to refer to the active substance liberated in the skin under certain conditions as the "H-substance." Tissue extracts can, however, be fractionated to eliminate certain interfering substances and then subjected to a complete physiological analysis, from the results of which the presence or absence of the amine can be legitimately inferred. For example, a tissue extract thought to contain histamine may be fractionated by procedures discussed elsewhere in this review, so that the active material is concentrated in a clear neutral solution which does not contain appreciable amounts of inorganic ions or salts which might affect the heart or peripheral circulation. If a certain amount of this neutral solution consistently produces in the atropinized etherized cat, a fall in blood pressure indistinguishable in all details from that caused by a fixed dose of pure histamine, and if the solution causes contractions of the guinea-pig uterus, relaxations of the rat uterus and bronchospasms in guinea pigs, all of which are indistinguishable from the effects produced under similar conditions by the equi-depressor amount of pure histamine as calculated from the results on the atropinized etherized cat, the inference that the active material is histamine is justified. The effects of equi-depressor amounts of the extract and of pure histamine should also be similar in the rabbit and in other animals. If the chemical fractionation is not sufficiently complete the physiological effects of the histamine present may be considerably modified.

Recorded work on the distribution of histamine is summarized in the tables, pp. 382–385. Positive and negative results are included. This summary indicates the value of the isolation methods, and by

adopting criteria suggested in the preceding paragraph one may judge as to the validity of the results. In view of controversies which have developed, more extended treatment is accorded certain tissues.

A. Plant sources. The first isolation of histamine was performed, as previously stated, by Yoshimura (1909). His material was not fresh, putrefaction having occurred, and one might expect as a result considerable histamine to be present. Yoshimura examined a decoction from putrid soy beans and an extract of the beans themselves. In the first case histamine was not definitely identified but in the second case this was accomplished.

The classical isolation of histamine by Barger and Dale (1910) was from a vegetable source, ergot. The work of Kutscher in the same year (1910) provided confirmatory results. Later investigators, Raymond-Hamet (1928) and Forst and Weese (1926), examined commercial fluid extracts of ergot for histamine. Forst and Weese found that the histamine content varied from traces to 0.4 mgm. per cubic centimeter. While histamine is the chief depressor constituent of ergot it is not the principal oxytocic factor (Bourne and Burn, 1927).

B. Animal sources. In a communication of historic interest, Chittenden, Mendel and Henderson (1899) dealt with the physiological effects of certain protein dervatives. These substances produced a sudden lowering of blood pressure which was probably due to the presence of histamine. Earlier work of a similar nature was quoted by these authors.

In 1900 Osborne and Swale Vincent found that brain and nervous tissue extracts contained a depressor substance the action of which was not abolished by atropine. Three years later Vincent and Sheen (1903) made saline, alcohol and ether extracts of various organs, and concluded that all glandular tissues, and probably all animal tissues, contain both a pressor and a depressor substance. In 1915 Rogers, Rahe, Fawcett and Hackett studied aqueous extracts of various organs and noted that the non-coagulable portion generally contained a depressor substance which had an effect on smooth muscle, and which stimulated gastric secretion.

Other work, in which tissue extracts containing an unidentified depressor substance have been prepared, might be mentioned. An interesting sequel of such work was the claim of Macdonald (1925) that special virtues were possessed by certain liver extracts in the relief of "essential or idiopathic hypertension." It was shown by Best, Dale, Dudley and Thorpe (1927) that such extracts owed their physiological

Distribution of histamine and histamine-like substances

MATERIAL	AUTHORS	DATE	METHOD
Ergot	Barger and Dale	1910	Isolation as picrate
	Kutscher	1910	Isolation as picrate and as hydrochloride
	Forst and Weese	1926	Assay on isolated uterus
	Raymond-Hamet	1928	No direct evidence
Yeast	Myers and Voegtlin	1920	Diazo reaction-physiological reactions
Witte's peptone	Abel and Kubota	1919	Incomplete isolation with tests on blood pressure
	Hanke and Koessler	1920	Diazo reaction—commercial peptone contains histamine; aseptically prepared peptone does not
	Abel and Geiling	1924	Chemical fractionation with biological tests
	Clark	1924	Histamine absent—chemical fractionation with biological tests
Erepton	Abel and Kubota	1919	Incomplete isolation with tests on blood pressure
Egg albumin	Abel and Kubota	1919	Incomplete isolation with tests on blood pressure
Casein	Abel and Kubota	1919	Incomplete isolation with tests on blood pressure
	Hanke and Koessler	1920	By chemical fractionation none; diazo reaction indefinite; depressor substance present
Edestin	Abel and Kubota	1919	Incomplete isolation with tests on blood pressure
Albumoses	Nagayama	1920	Biological tests of chemically fractionated material

Tamari-shoyn	Yoshimura	1909	Incomplete isolation
Soy beans	Yoshimura	1910	Complete isolation
Rattlesnake venom	Essex and Markowitz	1930	One chemical test negative; depressor substance present
Pituitary and pituitrin	Abel and Kubota	1919	Isolation as picrate
	Cow	1919	Histamine absent by tests on mouse uterus
	Jackson and Mills	1919	Biological tests show histamine in commercial pituitrin
	Abel and Macht	1919	Biological tests
	Dudley	1920	Histamine absent as shown by comparison of chemical properties of histamine and pituitrin
	Abel and Nagayama	1920	Chemical and biological properties show histamine in small amounts in commercial pituitrin
	Hanke and Koessler	1920	Histamine absent—diazo reaction—uterus response
	Dale and Dudley	1921	Comparison of effects of hydrolysis on histamine and pituitrin—histamine present in small amounts
	Roca	1921	Chemical fractionation—diazo reaction—uterus test
	Spiro	1922	Histamine absent
	Hogben and Schlapp	1924	Biological tests
	Sharpey-Schafer and Macdonald	1926	Biological tests
Coffee	Bickel and van Eweyk	1927	Method not given
Urine—dog	Koch	1913	Isolated as the aurate and as the picrolonate from urine of parathyroidectomized dogs

Distribution of histamine and histamine-like substances—Concluded

MATERIAL	AUTHORS	DATE	METHOD
Secretin	Parsons	1925	Diazo reaction—insufficient amounts to account for activity
Gastrin	Keeton, Koch and Luckhardt	1920	Histamine and gastrin not identical by biological comparison
Blood—human	Hanke and Koessler	1920	Histamine absent—diazo reaction
	Harris	1927	Biological assay
Blood—dog	Phemister and Handy	1927	Biological response shows a vasodilator substance in laked blood
Skin—human	Harris	1927	Biological assay (blood pressure uterine skin wheals)
	Lewis and Harmer	1927	Skin reactions
	Kalk	1929	Biological assay
Intestinal mucosa—ox	Barger and Dale	1911	Isolation as picrate
Intestinal mucosa—guinea-pig	Koessler and Hanke	1924	Diazo reaction
Intestinal mucosa—dog	Abel and Kubota	1919	Isolation as picrate
Intestinal contents—human	Meakins and Harington	1921	Partial fractionation—diazo reaction—uterus test
Intestinal contents—dog	Gerard	1922	Diazo reaction—blood pressure response
	Koessler and Hanke	1924	Diazo reaction
	Dragstedt	1928	Biological comparison
	Ingvalden, Whipple, Bauman and Smith	1924	Partial fractionation—diazo reaction
Intestinal contents—guinea-pig	Koessler and Hanke	1924	Diazo reaction
	Mellanby and Twort	1912	Biological comparison
Gastric mucosa—dog	Abel and Kubota	1919	Isolation as picrate
Feces—human	Watson	1928	Blood pressure response
	Koessler and Hanke	1924	Diazo reaction
	Eppinger and Gutman	1913	Isolation as the platinate

Feces—dog	Koessler and Hanke	1924	Diazo reaction
Muscle—human	Zuntz	1919	Isolation of the picrate from gangrenous muscle
Muscle—dog	Abel and Kubota	1919	Incomplete isolation
Muscle—horse	Thorpe	1928	Isolation as picrate
Lung—human	Peserico	1930	Biological comparison
Lung—ox	Best, Dale, Dudley and Thorpe	1927	Isolation as picrate
	Major and Weber	1929	Blood pressure response
Lung—guinea-pig	Rigler	1928	Blood pressure response
Liver—human	Koessler and Hanke	1924	Diazo reaction
Liver—dog	Koessler and Hanke	1924	Diazo reaction
	Lucas	1926	Diazo reaction
Liver—cat	Vincent and Curtis	1926	Biological comparison
Liver—dog	Abel and Kubota	1919	Isolation as picrate
Liver	Major and Weber	1929	Blood pressure response
Liver—guinea-pig	Koessler and Hanke	1924	Diazo reaction
	Rigler	1928	Blood pressure response
Liver—ox	Best, Dale, Dudley and Thorpe	1927	Isolation as picrate
Liver extract—beef	Burnett	1927	Partial chemical fractionation
Heart—calf	Oppenheimer	1929	Biological comparison
Heart—ox	Thorpe	1930	Isolation as picrate
Brain	Major and Weber	1929	Blood pressure response
Brain—cat	Vincent and Curtis	1926	Biological comparison
Spleen—ox	Dale and Dudley	1929	Isolation as picrate
Spleen—horse	Dale and Dudley	1929	Isolation as picrate
Sputa—human asthmatic	Knott and Oriel	1930	Uterus test

activity largely to histamine and choline. The results of the recent work of Bennet and Drury (1931) indicate that liver extracts prepared in a somewhat similar manner may also contain appreciable amounts of adenine compounds, which may produce a transient fall in blood pressure by dilating the arterioles. The favourable clinical results are probably attributable to rest and the psychic effects of treatment, both of which may profoundly reduce the height of blood pressure in these cases. When these factors were controlled the liver extract was found to be without therapeutic effect.

The first isolation of histamine from an animal source was that of Barger and Dale (1911) from intestinal mucosa. In 1919 a discussion as to the presence of histamine in the posterior lobe of the pituitary began. In that year Abel and Kubota made the claim that histamine was the active uterus-stimulating factor; the main constituent of "pituitrin." The claim was based on the isolation of histamine as the dipicrate from dried pituitary material. There can be no doubt that they actually secured histamine, but the amount was too small to account for the physiological activity of the material examined. Cow (1919) reported that the isolated uterus of the virgin mouse was relaxed by histamine and contracted by pituitary extract. Abel and Macht (1919), in reply to this, stated that Cow had used high concentrations of histamine, which poisoned the uterus. They found that the mouse uterus was affected similarly by weak solutions of histamine and of pituitary extract, but that these substances caused the rat uterus to react as had been previously reported by Cow. Using the same basis of physiological comparison, Jackson and Mills (1919) concluded that while histamine might be present in commercial pituitary extracts, the amounts were much too small to account for the oxytocic effects. In a paper in which they criticized the work from Abel's laboratory, Hanke and Koessler in 1920 stated that they had been unable to find histamine in extracts of fresh pituitary material. They suggested that Abel's results might be explained by bacterial formation of histamine in the glands after removal from the animals. This may have been so, but in later work, using fresh material with aseptic precautions, Abel secured histamine. The explanation of the failure of Hanke and Koessler to detect histamine is not apparent. They employed their isolation procedure, which involves several precipitations and extractions, before the application of the modified diazo reagents. If such a method indicates that histamine is present the results may be accepted, but if histamine is not found the possibility that it might have been present in the original material and lost in purification must be considered. Negative results secured by

Hanke and Koessler on pituitary and other tissues are therefore open to question. Dudley (1920) after comparing the chemical properties of histamine and of "pituitrin," concluded that histamine was not the active constituent of "pituitrin." The same year, 1920, Abel stated that he concurred in this conclusion (Abel and Nagayama) but he still believed that histamine was present in small amounts in pituitary extracts. In a later paper from Abel's laboratory (Roca 1921) histamine was found to be present in "pituitrin." Dale and Dudley summarized the evidence in 1921 and concluded that "histamine, if present at all, occurs in such minute amounts that its chemical identification is impracticable and there seems no reason at all for suggesting a relation between histamine and the specific action of the extract." Later workers have disagreed on this question. Spiro (1922) found none; Hogben and Schlapp (1924) and Sharpey-Schafer and Macdonald (1926) decided that it was present.

Summing up the evidence regarding the presence of histamine in pituitary extracts it may be stated that while it probably is present in very small amounts, it is certainly not a specific factor. If more definite evidence on this latter point were needed it has been supplied by Kamm, Aldrich, Grote, Rowe and Bugbee (1928) by their separation and purification of the active constituents of "pituitrin" preparations.

The paper in which Abel and Kubota (1919) claimed that histamine was the uterus-stimulating factor of the posterior lobe of the pituitary was of a most sweeping nature. Histamine it was stated was not only a constituent of the pituitary gland, but was also contained in intestinal and gastric mucosa, liver, striated muscle, erepton, Witte's peptone, egg albumin, casein and edestin. For intestinal and gastric mucosa the evidence was satisfactory; histamine was isolated as the picrate. The same could not be said for the other sources. Nevertheless, it now seemed to Abel that histamine provided a possible explanation for traumatic shock, intestinal intoxication, anaphylaxis and peptone shock. The possibility of the presence of histamine in so-called pure protein was at once considered by others. Using the diazo reaction Hanke and Koessler (1920) found 3.4 mgm. histamine in 100 grams of Witte's peptone. Hanke and Koessler prepared a peptone aseptically and were unable to find histamine in it by their procedure. Nevertheless, their peptone produced typical shock in guinea pigs and they concluded that, while histamine might be present in commercial peptone, histamine-free peptone produced shock. This pure peptone contained a substance (or substances) apparently basic in character, that was not destroyed by boiling with hydrochloric acid, was precipitated by phosphotungstic

acid, was extracted by amyl alcohol from an alkali aqueous solution, and combined with sulphuric acid to give salts that were soluble in water. The substance produced lowering of blood pressure. In these respects it was similar to histamine but it did not give the diazo reaction, nor did it contract the isolated guinea-pig uterus. Hanke and Koessler were unable to find histamine in pure casein but they did find that hydrochloric acid hydrolysis of casein yielded a solution which depressed blood pressure in a manner similar to histamine, but did not give the diazo reaction. In 1924 Abel and Geiling prepared sterile peptone, and as a result of a combination of chemical fractionation and physiological tests decided that it contained histamine. The identification was not completed. The same year Clark (1924) using methods similar to those adopted by the previous workers was unable to find histamine in peptone.

Histamine shock simulates peptone shock in many respects but not in regard to alteration of the clotting time of the blood. Acid hydrolysis of peptone and of some proteins yields extracts which behave pharmacologically like histamine solutions. The possibility has been suggested that histamine is formed during the hydrolysis, but that is quite unlikely. It is difficult to believe that pure proteins contain histamine, and there is unsatisfactory evidence regarding the nature of the depressor substances secured from them by hydrolysis.

Histamine has been definitely isolated from normal animal tissues. Best, Dale, Dudley and Thorpe (1927) showed that it was present in normal ox liver and lung. The base was secured as the picrate and the identity established. Using the same technique Dale and Dudley (1929) secured it from ox and horse spleen, and Thorpe (1930) from ox muscle and from the ventricles of beef heart. In addition to these results others have reported histamine in normal tissues, but in most cases the conclusion was based on results obtained with the diazo reaction or on physiological evidence, and not on complete identification. Zunz (1919) secured histamine as the picrate from human gangrenous muscles. Abel and Kubota (1919) did not complete the identification of histamine as a constituent of liver and muscle. From physiological evidence Major and Weber (1929) considered histamine to be present in liver, lung and brain. Koessler and Hanke (1924) using the diazo reaction found no histamine in human liver, or guinea-pig liver, but did find it in dog liver. With the same method Lucas (1926) found histamine in normal dog liver and in suprarenalectomized dog liver.

The possibility of the presence of histamine in blood is of interest. Koessler and Hanke (1924) were unable to find any in human blood.

Phemister and Handy (1927) reported that laked or traumatized dog blood contains a depressor substance which is not demonstrable before laking, and that the substance does not appear to be histamine. On the basis of physiological assay only, with no chemical evidence, Harris (1927) stated that blood contains 0.5 mgm. of histamine per kilogram.

The work of Sir Thomas Lewis (1927) on skin reactions leads to the conclusion that there is present in human skin an "H-substance" which strikingly resembles histamine. Harris (1927) estimated, from physiological assays, that a sample of normal skin contained 10 mgm. histamine per kilogram. Kalk (1929) has also suggested, as a result of his work on dermographia, that histamine is present in skin. The chemical isolation of histamine from this source has not yet been accomplished.

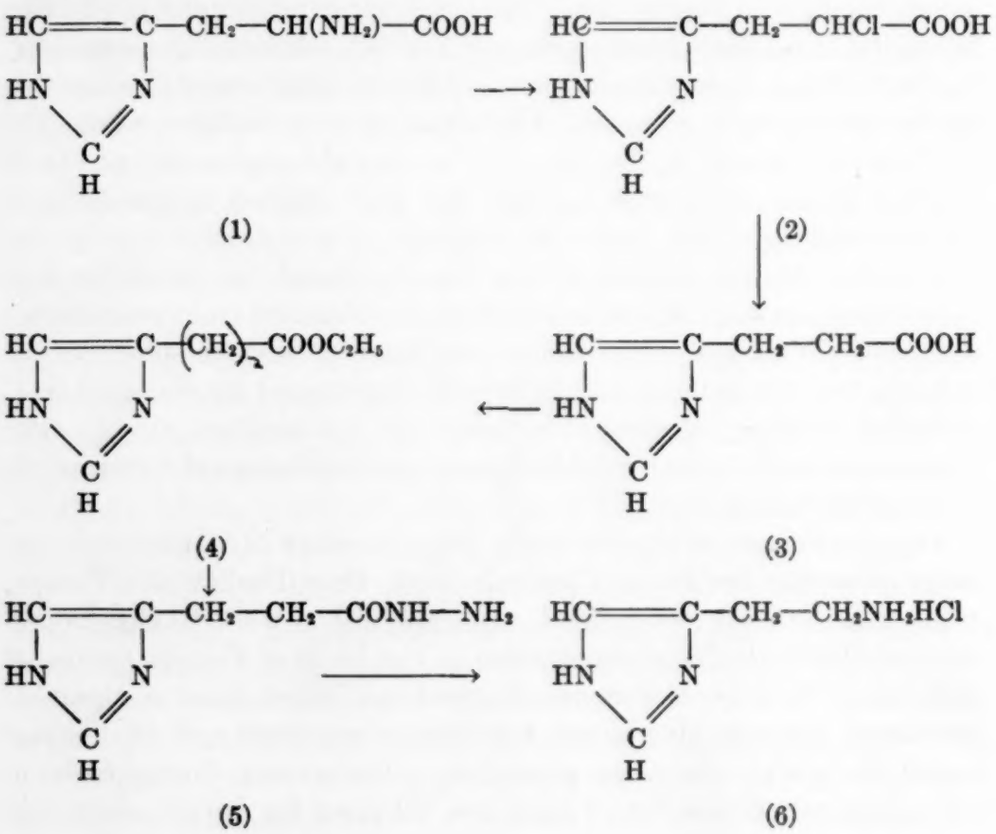
The possible presence of histamine in gastrin and secretin preparations is discussed elsewhere in this review.

Essex and Markowitz (1930) have recently published a series of papers on the pharmacological properties of crotalin, the venom of the rattlesnake. Crotalin behaves in some respects like histamine. It lowers blood pressure in etherized dogs and cats, contracts the isolated virgin guinea-pig uterus, produces bronchospasm in guinea pigs and causes the flare in human skin. On the other hand crotalin produces a fall in the blood pressure of an etherized rabbit, while histamine usually, but not always, causes an increase. The two substances produce dissimilar post-mortem pictures. Crotalin acts as an antigen, affects the erythrocyte volume in the dog and impairs the contractile power of skeletal or cardiac muscle. From the very limited examination of crotalin which we have made, the presence of more than a trace of histamine would not be suspected. On the other hand, the possibility that appreciable amounts of histamine might be obtained from crotalin has not been eliminated. The differences between such a substance as crotalin and histamine would be readily determined by chemical fractionation and physiological study, such as that outlined above. The situation is similar with regard to the presence or absence of histamine in many other materials.

The significance of the relatively large amounts of histamine in the lungs of certain herbivorous animals (Best, Dale, Dudley and Thorpe, 1927) has not been determined. In unpublished work Raxlen has estimated the histamine concentration in the lungs of various species of animals. The animals were anesthetized and killed under comparable conditions and the lung tissue was always extracted and the extract tested by exactly the same procedure. The average histamine concentration varied from 2 to 3 mgm. per kilogram for the rat and mouse

to approximately 40 for the guinea pig, ox, and horse. The lungs of the cat, chicken, rabbit and dog showed intermediate average values, from 8 to 28 mgm. per kilogram. Several groups of investigators have shown that the histamine concentrations of the two normal lungs of cats are the same within the limits of experimental error. Recently Macgregor and Peat (1931) have attempted to vary the histamine content of cat's lung *in situ* by stimulation of the vagus or sympathetic nerves and by the intravenous administration of epinephrine, pilocarpine or physostigmine. None of these procedures produced any detectable change in the amount of histamine present. Rigler (1928) has compared the amounts of vasodilator material in the lungs and livers of normal and sensitized guinea-pigs. The organs of the sensitized animals appeared to contain less histamine-like substance than those of the normal pigs, but the figures are unfortunately not convincing.

SYNTHESIS. Some three years before any physiological significance was attached to histamine, or before it had been isolated from a biological source, it had been prepared by Windaus and Vogt (1907). The procedure was a complicated decarboxylation of histidine and is represented as follows:

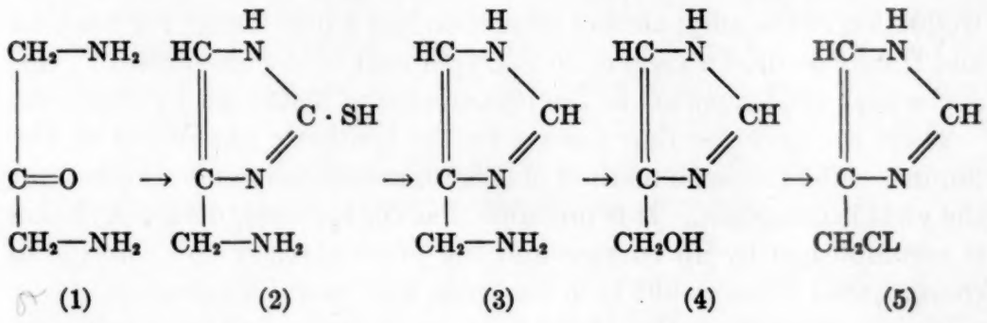


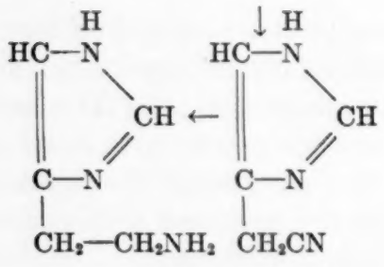
By the use of sodium nitrate and hydrochloric acid, histidine (1) is converted into α-chloro-β-iminazolylpropionic acid (2), which is reduced to β-iminazolylpropionic acid (3). This acid is esterified (4), and the ester changed to the hydrazide (5). Amyl nitrate and hydrogen chloride in alcoholic solution convert the hydrazide to the azide and urethane; hydrolysis of the urethane with concentrated hydrochloric acid gives histamine hydrochloride (6).

This circuitous method is necessary because it is difficult to decarboxylate histidine directly by chemical means. In 1910 Ackermann described the direct decarboxylation by bacteria. This is discussed in the section on bacterial formation of histamine.

In 1911 two papers were published from the Burroughs-Wellcome laboratories dealing with histamine synthesis, the first by Ewins and Pyman, the second by Pyman. The first paper was concerned with the direct, chemical decarboxylation of histidine. Direct heating of the amino-acid in sealed tubes at temperatures up to 270°C. gave a 1 per cent or smaller yield of the amine. Direct heating of the monobenzoyl derivative of histidine with subsequent hydrolysis gave a 10 to 20 per cent yield. The yield could be increased to 25 per cent by heating histidine with concentrated hydrochloric acid, 20 per cent aqueous sulphuric acid, or fused potassium hydrogen sulphate at a temperature between 265–270°C. Below or above this temperature range the yield was decreased. The use of 44 per cent phosphoric acid, or hydrogen bromide in acetic acid solution did not give a better result. The direct chemical decarboxylation of histidine has not yet proved a good method for preparing histamine.

In the second paper Pyman described the synthesis of histamine from diaminoacetone. This compound was prepared from citric acid. The procedure for the preparation of histamine was as follows:





(7) (6)

In this process the iminazole ring is formed by heating diaminoacetone (1) with potassium thiocyanate, which produces 2-thiol 4 (or 5)-aminomethyliminazole (2). This is oxidized with nitric acid to give aminomethyliminazole (3), but during this reaction nitrous acid is liberated and it causes the formation of hydroxymethyliminazole (4). The action of phosphorus pentachloride on this gives chloromethyliminazole (5), which with potassium cyanide yields cyanomethyliminazole (6). This is reduced with sodium and alcohol to give histamine (7). During the process a number of side reactions take place; these produce compounds other than those desired and reduce the yield. Using the outlined procedures Pyman reported a yield of 29 per cent of the theoretical. The process gives more iminazolylacetic acid (35 per cent of theoretical) than it does of the desired base.

Koessler and Hanke in 1918 followed, in general, Pyman's method, but introduced some modifications. The most important change was in the reduction of cyanomethyliminazole. This was again accomplished with sodium ethylate, and the histamine, instead of being precipitated as the picrate, was extracted from the alkaline mixture with amyl alcohol. The combined amyl alcohol solutions were extracted with dilute hydrochloric acid. Colouring matter was adsorbed on charcoal and histamine hydrochloride crystals were secured. The introduction of the amyl alcohol extraction was a useful step, for Koessler and Hanke secured a yield of 50 to 56 per cent of the theoretical. This is the best yield reported in the preparation of histamine by synthesis.

There are only the four reports on the synthetic production of histamine. The bacterial method of Ackermann seems much simpler, but the yield is uncertain. It is probable that the bacterial decarboxylation is accomplished by an enzyme and the preparation of this enzyme in concentrated form would be a desirable and useful attainment.

Within recent years there have been several papers on the production of histamine from histidine by ultra-violet irradiation. Ellinger in

1928 showed that irradiation of histidine solutions produced a substance which resembled his tamine pharmacologically. Only about one five-hundredths of the histidine was decarboxylated. The transformation was, to some extent, independent of the pH and of the period of irradiation, but when the acidity was high the reaction did not occur. Substances such as eosin and ferric chloride did not augment the yield, but this was influenced by the wave-length of the radiations. Irradiation of serum failed definitely to increase the quantity of histamine-like substance present, but Ellinger believed that extracts prepared from areas of irradiated skin in some instances showed a greater activity than did extracts from normal skin. If such were the case it could be as well explained by supposing that preformed histamine was liberated from cells by the irradiation, as by the hypothesis that histamine was formed by irradiation.

Bourdillon, Gaddum and Jenkins (1930) repeating Ellinger's work found that histamine was produced by the ultra-violet irradiation of histidine, but by energy of wave-length less than 265$\mu\mu$. These authors held that Ellinger's hypothesis of histamine formation in irradiated skin was unproven and that it was more likely that the irradiation liberated preformed histamine.

Ellinger (1930) has extended his work by showing that histamine is formed whether the histidine, during irradiation, is in solid form or is in solution. Moreover, he observed that irradiation of histamine in solution would inactivate it. During the irradiation of histidine there is produced a yellow colour which acts as a screen, filtering out the energy which would inactivate the histamine, and thus protecting the base as it is formed.

THE BACTERIAL PRODUCTION OF HISTAMINE. Investigation of physiologically active basic substances, given the name ptomaines, began with the extraction of these bases from putrefying flesh. These substances, in many cases, were found to be amines derived from naturally occurring amino-acids by the removal of the carboxyl group. Such a reaction is accomplished, by chemical means, only with considerable difficulty, as was found when attempts were made to prepare histamine from histidine directly. Under certain conditions, however, this reaction is readily effected by bacterial action.

Two of the most familiar ptomaines, cadaverin and putrescin, are derivatives of amino-acids. Lysin, on decarboxylation, yields cadaverin, and diamino valerianic acid gives putrescin. Similarly tyramine is derived from tyrosine, and histamine from histidine.

In 1910 Ackermann prepared histamine by the action of putrefactive micro-organisms on histidine. He stated that the method he employed, and which he advocated as a means of preparing histamine in quantity, gave a good yield, but other investigators have found that unless the method is very closely followed the yield is low. Ackermann dissolved 49 grams of histidine dichloride in 4 litres of water. He added 10 grams of Witte's peptone, 20 grams glucose, a few drops of solutions of magnesium phosphate and of sodium phosphate, and an excess of calcium carbonate to prevent the solution from becoming acid. The medium was inoculated with a little finely minced ox pancreas which had previously been incubated for twenty-four hours in a weakly alkaline medium, and the whole was kept for fifty-two days at 35°C. After this period saturated picric acid solution was added to precipitate histamine as the dipicrate, which is sparingly soluble in cold water and can be crystallized from hot water. From the picrate the histamine was recovered by adding hot dilute hydrochloric acid and extracting the picric acid with ether. The hydrochloride of the base remained in the aqueous solution. The yield was approximately forty per cent of the theoretical.

As a result of Ackermann's work two patents were granted for the production of histamine from histidine by bacterial action, one to Hoffman, LaRoche and Company (1912), and the second to Farbenfabriken von F. Bayer.

Two years after Ackermann's work, Mellanby and Twort (1912) reported the identification of histamine as a constituent of intestinal mucosa, and the isolation of a bacillus from intestinal contents which had the power of converting histidine into histamine. This work, like most of the subsequent study of bacterial production of histamine, was an outgrowth of a study of "intestinal intoxication."

Mellanby and Twort isolated from the feces, presumably, although not definitely stated, of guinea pigs, a bacillus which formed histamine. The presence of the amine was indicated by its effects on the isolated guinea-pig uterus. The bacilli from the feces were subcultured in various media to secure the strain most active in producing histamine. This strain was then grown for twenty-four hours on glycerin-agar and subsequently transferred to a solution containing histidine and inorganic salts. The production of the amine in this way is not difficult to carry out in test tube lots. For large scale work it is necessary 1, to inoculate the solutions with a very vigorous culture, and 2, to inoculate with a correspondingly large number of bacilli when large quantities of medium are used.

In contrast to Koessler and Hanke, whose work will presently be described, Mellanby and Twort found that the bacillus which produces histamine under certain conditions grows well in acid media but histamine is *not* produced. At 37°C., in neutral solution histamine was secured but at 45°C. no amine was obtained. The bacillus was said to belong to the colon-typhoid group and to have the following characteristics: a small bacillus with rounded ends, non-motile and Gram-negative. It grew aerobically or anaerobically on the ordinary laboratory media. The growths on gelatine, agar and broth were similar to those of *B. coli*. Milk was clotted and no liquefaction of gelatine took place. Acid and gas were produced in media containing glucose, lactose or dulcite.

Shortly before Mellanby and Twort's publication, Barger and Dale (1911) had isolated histamine from intestinal mucosa. Since the bacillus which decarboxylated histidine was present in the alimentary canal from the duodenum downwards, at least in guinea pigs, Mellanby and Twort believed that the presence of histamine in the intestinal mucosa was due to bacterial decomposition occurring in the intestinal contents.

Berthelot and Bertrand (1912), almost coincidently with Mellanby and Twort, isolated from human intestinal contents a bacillus which produced histamine from histidine. The organism was very similar to *B. pneumoniae* and to *B. lactis aerogenes*, but these latter, even after passage through several media will not decarboxylate amino-acids. To the organism which accomplished this Berthelot and Bertrand gave the name *B. aminophilusintestinalis*. This bacillus will ferment certain sugars and will act on histidine, tyrosine and tryptophane. Depending on the medium, the amino-acids are decarboxylated or deaminized. Between *B. proteus* and *B. aminophilus* there appeared to be a symbiotic relationship, which rendered the combination highly effective. Berthelot and Bertrand demonstrated the presence of histamine by characteristic physiological reactions, such as the production of bronchospasms in guinea-pigs, and by isolation of the base as the picrate.

A paper, largely speculative, by Mutch (1914) contained a report of the isolation of histamine from the feces of patients suffering from chronic constipation. Samples of ileum and of the contents of the ileum were removed during operations. The last coil of the ileum was reported to yield a strain of *B. coli* capable of converting histidine to histamine. Other parts of the ileum apparently yielded few, if any, aerobic bacteria.

The question of the bacterial production of histamine then lay dor-

mant for several years, but the preceding work had laid the foundation for the extensive studies reported by Koessler and Hanke between 1919 and 1924 and reviewed by them in 1928. The first paper (1919) in their series, dealt with the preparation of the necessary media. Contrary to Mellanby and Twort, Koessler and Hanke found that histamine is produced only in media in which acid has been formed by fermentation of glycerol or sugar. They suggested that the formation of the amine is a protective mechanism for the maintenance of neutrality.

In paper XVII Koessler and Hanke (1924) reported that of 26 human stools examined, 16 (62 per cent) contained a micro-organism which decarboxylated histidine, 17 (65 per cent) decarboxylated tyrosine, and 12 (46 per cent) produced both histamine and tyramine. Eighteen of the 26 stools were from normal individuals.

The extensive work of Koessler and Hanke has been well summarized in a recent paper (Koessler, Hanke and Sheppard, 1928). Reviewing the ten years' work the authors again emphasize the point that they never observed the production of histamine and tyramine excepting in a medium which had become acid. The power of decarboxylating amino-acids is not common to many bacteria, and it cannot be correlated with any of the ordinary cultural characteristics. This property seems to be constant in Gram-negative organisms. No single micro-organism has yet been isolated which will decarboxylate both histidine and tyrosine, possibly because the chemical mechanism involved is not the same in each case. Decarboxylation is a fairly common property of the mixture of micro-organisms found in human feces. More than 50 per cent of all whole-stool inoculations accomplished a production of histamine, or tyramine, or both. The authors raised the interesting point, previously indicated by Berthelot and Bertrand, that the decarboxylation is due to the symbiotic activity of a mixture of bacteria. The mixture of bacteria in the sputum, as a rule, does not produce either histamine or tyramine. The recent report of histamine-like substances in sputa from asthmatic patients (Knott and Oriel, 1930) is interesting in this connection. These investigators report that bacilli which produce histamine-like substances when they are grown in broth culture, can be recovered from the sputa of these cases.

In the more recent work discussed in their paper, Koessler and Hanke report the production of a spastic substance (or substances), amine in nature, the isolation of which has not been completed. It does not appear to be identical with histamine. This is another example of the occurrence of a substance which causes physiological effects strikingly

similar to those produced by histamine, but apparently due to some other, perhaps more complex, substance.

In their summary Koessler and Hanke discussed the results of a study of 223 micro-organisms, grown on a blood-broth medium. Ninety-four were members of the colon-typhoid group, of these 9 converted histidine to histamine, 2 being *Escherichia*, and 7 *Salmonella*. Five of 8 *Salmonella morgani* strains produced histamine. There were no tyramine producers in this group. The ability to produce a bronchospastic and arteriospastic substance, apparently not histamine, is stated to be highly developed in the colon-typhoid group. Of the 94 representatives of this group studied, 67 produced spastic substances. Histamine was not formed by any of the 129 micro-organisms that are not members of the colon group, but of these, five, all streptococci, were tyramine producers.

Kendall and Schmitt in 1926 examined 72 strains of *B. welchii*, isolated from the stools of 41 persons, and noted that 66 of the 72 strains caused the production, in the media containing carbohydrate, of a soluble poison which had two definite physiological effects: 1, contraction of isolated guinea-pig intestine; 2, the production of an immediate transitory fall in the blood pressure of an etherized cat. Further work on the identity of the poison was not performed, but it may be observed that these two effects are characteristic of histamine and that the results are comparable to those of Koessler and Hanke.

Roske (1928) studied the production of amines by *B. coli* in artificial media. In peptone broth which becomes alkaline on incubation, no amines are formed. On the other hand, in dextrose broth which becomes acid on incubation, large quantities of amine are produced. Buffering the dextrose medium to prevent an increase of acidity prevents the formation of amines. This supports the hypothesis of Koessler and Hanke that the production of amines by the bacilli may be a protective mechanism against acid.

Noting that persons with chronic infections may have low blood pressures, Squier and Bach (1928) tested the products of cultures of organisms from human teeth, tonsils and prostatic secretion on the blood pressure of rabbits. The pressure was lowered and it was suggested, from very scanty evidence, that the depression might be due to histamine. Several other groups of clinical observers have suggested, without adequate experimental evidence, that since organisms obtained from foci of infection in the body may produce histamine under certain conditions *in vitro*, the same process may be taking place when they grow in the body.

Practically all the work on bacterial production of histamine has been for the purpose of securing information on so-called "intestinal intoxication." The study is far from complete but there is definite proof that strains of *B. coli*, commonly found in human and other feces, decarboxylate histidine to form the toxic amine. The hypothesis of Koessler and Hanke that this production of base is a protective mechanism against acids formed from sugars in the medium, has been supported. The histamine which has been isolated from intestinal contents probably results from intestinal putrefaction, but no proof is yet available that this histamine is an agent in the production of the signs or symptoms of "intestinal intoxication."

THE PRESENCE OF HISTAMINE IN THE ALIMENTARY TRACT. The report of the isolation of histamine from intestinal mucosa (Barger and Dale, 1910) is the first record of the preparation of the amine from animal tissue. The mucous membrane of fresh gut was removed, ground with sand, and extracted with dilute hydrochloric acid. After boiling, the mixture was filtered and the filtrate concentrated. The precipitate produced by 20 per cent tannin was removed. The filtrate was treated with excess of barium hydroxide in the presence of silver nitrate according to Kutscher's method. The bases were regenerated from the silver precipitate and extracted with hot absolute alcohol. The alcohol was removed by evaporation and the syrupy residue yielded a crystalline picrate on treatment with a saturated solution of picric acid. This salt was identified both chemically and physiologically as the dipicrate of histamine. It is probable that some of this histamine was present in the mucous membrane of the intestine and was not formed by bacterial action but the possibility of bacterial formation was not excluded in these experiments.

Eppinger and Gutman (1913) in the first report on the isolation of histamine from human feces stated that they had obtained small amounts of the base as a picrate and platinate from the feces of normal people and that the quantity present in the feces of patients suffering from intestinal disorders was considerably greater.

Abel and Kubota (1919) isolated histamine in crystalline form from the mucosa of dog stomach and small intestines. They also obtained histamine in the form of a slightly impure picrate from the upper portion of fresh pig intestine. Although the authors believe "that the greater part of the base, if not all of it, must have been produced by non-bacterial hydrolytic processes" the possibility exists that the amine may have been formed by the action of bacteria in the intestine before the death of the animal.

In their tests for the presence of histamine in the human intestine, Meakins and Harington (1921) fractionated the material from the patients in such a manner that the possibility of the presence of interfering substances was very slight. The material, as soon as possible after collection, was treated with a solution of mercuric chloride and HCl. The mixture was filtered and the clear filtrate was freed from excess of mercury by means of hydrogen sulphide. The mercuric sulphide was removed and lead acetate was added. After removal of the precipitate and the lead the solution was tested on the virgin guinea-pig uterus. By this method the presence of minute amounts of histamine was demonstrated in the cecum and transverse colon of several cases. Histamine was found in the unobstructed human intestine. These authors found none in the feces but other investigators report positive results.

Gerard (1922) impressed by the similarity between the properties of certain constituents of the fluid which accumulates above an obstruction of the small intestine in experimental animals and those of histamine, studied the contents of closed loops of intestine. He did not isolate histamine in crystalline form but found a substance with many of the chemical and physiological properties of this amine. He records the presence of this substance in sterile loop fluid. Gerard obtained some evidence that histamine is present in these loop fluids in a combined as well as a free form. An average amount of histamine or histamine-like material found was the equivalent of 2 to 3 mgm. of the dichloride per 100 cc. of the loop fluid.

Hanke and Koessler (1924) have applied their method of estimation of histamine to human cecal contents and feces. Colour values corresponding to as much as 20 mgm. of histamine from 648 grams of moist feces were obtained. Smaller amounts were thought to be present in cecal contents. The presence of histamine was also indicated in dog feces, but the intestine of the guinea pig yielded no substance giving the characteristic colour reaction.

Ingvaldsen, Whipple, Bauman and Smith (1924) concluded from their work on the nature of the toxin in intestinal obstruction that although the loop fluid contained some histamine there was not enough present to account for the toxic effects. The details of their colorimetric test for histamine are not given. The results of the recent work on histamine indicate that some of the amine may have been lost in the various chemical processes to which the loop fluid was subjected.

Dragstedt (1928) in a study of the blood chemistry in intestinal obstruction emphasizes the similarity between the effects of injection of

obstruction fluids and of histamine. In dogs the rise of non-protein and urea nitrogen, the fall of blood chlorides and the splanchnic engorgement are similar in the two cases. The toxic fractions are powerful stimulants of gastric, pancreatic, and intestinal secretion, as is histamine. This work in conjunction with that of other investigators suggests that histamine is one of the toxins found in infected obstruction fluids.

Gatch, Trusler and Ayers (1928) found filtrates from the contents of obstructed intestines in dogs to be toxic on injection into normal animals. They also report that 250 cc. of blood from a dog dying of intestinal obstruction when injected intravenously into a normal dog, from which an equal quantity had been removed, produced a temporary violent reaction similar to histamine poisoning. Intestinal cramping, vomiting and purging, respiratory distress and circulatory collapse were observed. This experiment is interesting but was inadequately controlled, as the blood of a normal dog is not always compatible with that of others.

The absorption of histamine from the alimentary tract. Mellanby (1915) found no disappearance of histamine from the small intestine of cats when the blood vessels supplying the part were ligatured. He obtained the maximum rate of absorption at the lower end of the small intestine. Mellanby did not observe any absorption of the histamine from the large intestine. He attributed the disappearance to bacterial action, but Meakins and Harington, also using cats under anesthesia, found a definite absorption of histamine from the large intestine. According to Mellanby the absorption of histamine from the small intestine is modified by a variety of factors. The presence of food such as milk or meat or fat delayed absorption. Ringer's solution also delayed the disappearance of the amine. Just how firmly the histamine may have been bound to the various food materials and the part this might have played in the effects secured is difficult to decide. Alkali appeared to increase and acid to diminish the absorption. Mellanby observed the important effect of altering the volume of body fluid on the absorption of histamine. When the volume of fluid is decreased the amine is absorbed less rapidly, but the body has little power of resistance. The resistance to the amine was greatly increased when an abundance of fluid was provided.

Popielski (1920) found no effects of histamine injected into the duodenum of dogs but when the injection was made into the mesenteric veins about the same fall in blood pressure was obtained as when the material entered by a peripheral vein. He infers from these results that histamine is destroyed during its passage through the wall of the small

intestine. Meakins and Harington (1922) using cats under paraldehyde anesthesia, found that the rate of absorption of histamine was greatest from the ileum, somewhat less from the cecum and stomach. Experiments with Eck fistula animals suggested that the liver might act as a trap for histamine by retaining some of the amine in the blood which is held in the engorged vessels of the liver. This phenomenon was observed in our liver perfusion experiments and to a smaller degree in the perfusion of other organs. Ivy and Javois (1924) could find no difference between the tolerance of normal and Eck fistula dogs to histamine given by stomach tube. These investigators regard these results as proof that histamine is inactivated in its passage through the intestinal wall. They have given as much as 750 mgm. to normal dogs and 225 mgm. to man without the production of severe symptoms. Mutch (1914) in a less convincing paper has reported the production of toxic symptoms in man from relatively small doses.

McCarrison (1924) found that histamine fed to birds over long periods retarded the normal increase in weight. Ingestion of the amine produced a sharp fall in respiratory rate which recovered slowly but not to the normal value. After an initial rise body temperature was depressed. The thymus and thyroid glands were reduced in size but the parathyroids were unaffected. Histamine produced a definite change in the adrenal medulla of young pigeons but not in that of young rats. The adrenal glands of the rats were increased in size. Histamine caused a definite reduction in the weight of the heart in pigeons and rats. The weight of the lung was increased in young rats. Adult animals became habituated to the continual ingestion of histamine, since the effects on respiration and body weight became less marked. The effects of histamine on the growth of young animals persisted after ingestion of the amine was discontinued.

Koessler and Hanke (1924) found that small doses of histamine introduced into the mouths of guinea-pigs caused death. They state that incubation of histamine with the contents of the intestinal tract of guinea pigs does not render the amine unrecognizable. In one experiment they administered per os 100 mgm. of histamine to an 800 gram guinea pig. Only slight symptoms were produced. The animal was killed the following day and the intestinal tract analyzed for histamine. Only 1.6 mgm. were found. In a 5 kilogram dog they found that ingested histamine disappeared from the alimentary tract at the rate of 2.2 mgm. per minute. They found evidence of the absorption of only small amounts and conclude that the amine is rapidly inactivated during

passage through the intestinal wall. These experiments have been discussed in more detail under "Inactivation of histamine."

Wangensteen and Loucks (1928) found that absorption of histamine from the normal small intestine of the dog and in simple obstruction of the small intestine of two days' standing could not be detected by the physiological test for histamine. In strangulation obstruction a great fall in arterial blood pressure occurred following release of the strangulating mechanism. The autolysis of the intestinal mucosa, deprived of its blood supply, was rapid and was accompanied by the liberation of a toxic substance which gave an effect like that of histamine. Absorption of histamine from the lumen of a still viable strangulated segment was slight.

Mammoser and Boyd (1929) found that in small dogs under barbital anesthesia the application of chloroform in paraffin oil to the mucosa of the duodenum resulted in increased absorption of histamine. HCl, carbon tetrachloride, and ethyl alcohol also increased the absorption, sodium fluoride in 1 per cent solution gave negative results. Albi and Boyd (1929) could not demonstrate any effects of removal of the parathyroids on the absorption of histamine from the dog's intestine.

The effects of local application of histamine to the mucosa of the colon of decerebrated cats has recently been studied by Florey (1930). Histamine solution (1 mgm. per cc.) was injected through the anus, which was then closed by sutures. From 1½ to 2 hours later the colon was examined. There was very little demonstrable change in the mucosa. Stronger solutions of histamine were also ineffective in the production of mucus. Florey found little absorption of histamine, but a continuous fall of blood pressure. He concludes that histamine does not cause the goblet cells of the colon to secrete. Koskowski's observation (1926) that histamine caused the expulsion of a yellow plug, coated with fresh mucus from the Thiry fistula of a dog does not necessarily imply that the histamine caused secretion of mucus.

MacKay (1930) in a study of intestinal motility in cats, found that when 50 to 100 mgm. of histamine were introduced into an isolated loop of intestine no fall of blood pressure, no effect on intestinal motility, and no destruction or inactivation of histamine, were produced. The conditions controlling the absorption of histamine from the intestine are, obviously, inadequately known. Convincing evidence of absorption has undoubtedly been obtained by several investigators. The recent work of Fényes (1930) in which relatively small doses of histamine administered per os to human subjects are reported to produce definite

effects, is briefly discussed under "Action on human subjects." In a number of instances the effect on gastric secretion of application of histamine to various parts of the intestine has been reported. These studies will be considered under "Gastric secretion."

No attempt will be made to summarize completely these two sections, but since most of the work referred to was undertaken with the hope of gaining some information on the cause of the effects of intestinal obstruction, a few comments on the bearing of the observed facts on this subject may be made. It is established that histamine may be present in the mucosa and in the contents of the unobstructed bowel. In a closed loop of intestine in animals substances having many physiological and chemical properties similar to histamine accumulate. There is convincing evidence that histamine may be absorbed from the mouth and small or large intestine of animals and when administered orally to human subjects. The amount absorbed varies greatly in the different species and, as a general rule, relatively large quantities must be available to secure a rate of absorption which produces effects comparable to those observed after the subcutaneous or intravenous administration of very small doses. It can not be emphasized too strongly that absorption of histamine after the application of foreign substances or of unphysiological concentrations of naturally occurring materials to the intestine provides no evidence in favour of a physiological rôle of this mechanism. The development of increased pressure within the lumen of the gut may accelerate the absorption of histamine or similar substances. There are very few data on the effects of the prolonged oral administration of the amine to mammals. From the data reviewed here and from an examination of the literature on intestinal obstruction there appears to be no ground for supposing that histamine plays an important rôle in the production of the signs and symptoms of simple intestinal obstruction. The experimental evidence suggests that the signs of toxemia observed when the fluid which accumulates above an obstruction becomes heavily infected or when the bowel is strangulated may, in part, be due to the absorption of histamine or closely related substances. Conclusive proof of the participation of histamine in the production of these effects is lacking.

Histamine and peptic ulcer. Acute peptic ulcers can be produced experimentally in a great variety of ways. McIlroy (1928) observing an acute ulcer in the stomach of a cat which had received histamine subcutaneously found that extension of lesions artificially produced in the gastric mucosa took place when large doses of histamine were ad-

ministered subcutaneously to a series of cats. Büchner, Liebert and Molloy (1928) reported the production of ulcers in rats which had received histamine. O'Shaughnessy (1931) reports the production of acute ulcer, and in one case a more chronic type, by the local injection of histamine into the muscular layer of the stomach wall of cats. O'Shaughnessy has suggested that histamine liberation may be a common factor in the various methods of production of ulcer. It is obvious that there is not as yet sufficient well-controlled experimental evidence to justify more than the briefest reference to the possible significance of histamine in this field.

ACTION OF HISTAMINE ON DIGESTIVE GLANDS. *Salivary secretion.* In their study of the physiological properties of histamine Dale and Laidlaw (1910) noticed that salivation was one of the effects of intravenous administration of the base to unanesthetized cats or to cats under paraldehyde. Dogs anesthetized with an alcohol-chloroform-ether mixture also exhibited a secretion of saliva after histamine. In both the dog and the cat they found that the action of histamine on salivary secretion was not demonstrable after atropine. Fröhlich and Pick (1913) published tracings illustrating the stimulating effect on salivary secretion of intravenous histamine injections in decerebrate cats. A large dose of histamine may cause a decreased blood flow through the gland and repeated doses may "poison the cells" so that they do not react to stimuli which are normally effective. MacKay (1927) found that in dogs or cats under chloralose, ether or chloroform anesthesia, the administration of histamine by the intravenous, subcutaneous or intramuscular route was followed in about half the cases by a flow of saliva. When the effects of anesthetics were eliminated by using decerebrate cats the action of histamine was appreciably greater. Histamine after pilocarpine usually inhibited secretion, but if the chorda tympani had been stimulated previously histamine augmented the salivary flow. This latter effect was abolished by atropine. In another paper (1927) MacKay attempted to correlate these findings with vascular changes in the gland. She found that histamine in both cats and dogs caused an increase in blood flow through the salivary glands. The acceleration in the cat was long continued, but the transiently augmented flow in the dog was, in some cases, followed by a diminution. Atropine, in sufficient dosage to paralyze the secretory action of the chorda, did not affect the change in blood flow through the gland caused by histamine. The action of histamine after atropine was largely independent of blood pressure. The injection of histamine following pilocarpine in a cat caused a

diminution in the blood flow through the submaxillary gland. This reversal effect of pilocarpine on the action of histamine could also be demonstrated in the dog, but a much larger dose of pilocarpine was required. If atropine was given after pilocarpine the vasodilator effect of histamine was restored in both the cat and the dog, but the original level of blood flow was not attained. In commenting on MacKay's work, Ivy (1930) points out that histamine given intravenously in the doses MacKay used can not be considered to be within the physiologically possible range. When small doses are given subcutaneously to human subjects or to dogs no salivation is noticed.

MacKay suggested that histamine has a double action on the salivary gland: 1, a secretory effect increased by chorda stimulation, and 2, a mechanical effect due to a constrictor action on the smooth muscle of the gland. Osborne (1928) has more recently reached similar conclusions as a result of his experiments on dogs. He failed to produce salivary secretion when he lowered the systemic blood pressure by various procedures. Osborne reports that atropine produced great diminution but not an abolition of histamine effect on salivary secretion. In 1929 MacKay showed that paralysis of the sympathetic nerve supply to the submaxillary gland of the cat does not abolish, although it may diminish, the effect of histamine on salivary secretion. After both atropine and ergotoxin histamine apparently still has a slight action. In this work MacKay obtained evidence suggesting that histamine does not act on smooth muscle fibres in the submaxillary gland. In a further study of this problem Babkin and MacKay (1930) report that Bowie was unable to find any smooth muscle in the ducts or in any other part of the gland of cats, dogs or rabbits. The explanation of the motor phenomena produced in the gland by histamine is not available. The dilatation of the blood vessels might press out saliva, but the increase in blood flow and in the volume of the gland is apparently of short duration and the secretion of saliva is found to coincide with a period of inhibition of blood flow.

Gastric secretion. There are several references in the literature in regard to the effect of tissue extracts on gastric secretion (Tomaszewski, 1918), and it is probable that histamine was the active constituent. The action of histamine on gastric secretion was first studied by Popielski (1920). He administered histamine subcutaneously to dogs with gastric fistulae and noted the increased flow of gastric juice. Vagotomy or administration of atropine did not inhibit this effect and he concluded that histamine acts on the glands themselves and not through nerve

stimulation. He recognized that the presence of histamine was responsible for many of the effects of organ extracts. In a second paper Popielski (1920) records, among others results, the effect of administration of 0.4 mgm. of histamine subcutaneously to one of his assistants. Although the gastric juice was not studied by Popielski he attributes many of the effects obtained to dehydration produced by gastric secretion. Keeton, Koch and Luckhardt (1920) found that histamine stimulated the gastric secretion of the dog, cat, rabbit, guinea-pig, duck and frog. They discussed the possibility that histamine was the active constituent of "gastrin" preparations but found certain differences when secretory and toxic effects of the two were compared. Koch, Luckhardt and Keeton (1920) noted that histamine added to gastric preparations was precipitated by picric or picrolinic acids but that the "gastrin" activity was not. In the light of recent knowledge it is possible that all the naturally occurring histamine was not precipitated under these conditions. Burgess, Sacks, and Ivy (1930) have found that when amounts of "gastrin" and histamine having equal effects on gastric secretion are given intravenously the depressor effect of histamine is much greater. It is not probable that histamine is so bound in the "gastrin" preparation that it can be slowly liberated so as to affect gastric secretion, but not blood pressure when injected intravenously. Necheles and Lim (1928) have reported the preparation of a stimulant of gastric secretion which has very little depressor activity. This question will perhaps be reopened by a continuation of the recent work of Sacks, Ivy, Burgess and Vandolah (1931) who have isolated histamine from fresh gastric mucosa. As Ivy has stated, histamine and "gastrin" may be very similar chemically. The possibility that they are identical still exists.

Rothlin and Gundlach (1921) were among the first to study the effect of histamine on gastric secretion. They found a rough parallelism between the quantity of gastric juice and the amount of histamine given subcutaneously to dogs. They noted that histamine acts much more powerfully on the cells which provide the fluid than on the enzyme producing mechanism. They did not secure positive results on gastric secretion when histamine was given intravenously or when small amounts were applied to the intestine.

Koskowski (1922) found that histamine, when given intramuscularly or subcutaneously, stimulated gastric secretion in pigeons but that the amine was inactive in this regard when given intravenously. Secretion was stimulated when relatively large doses were introduced into any part of the intestine. He reported that the blood removed from a

pigeon which had received histamine produced gastric secretion when administered subcutaneously to another pigeon. (His experiments on the stability of histamine in rabbit blood were not well-controlled.) Lim (1922) found that the gastric glands were stimulated slightly when from 1 to 2 cc. of a 0.0001 per cent histamine solution were injected intravenously into anesthetized cats. Ivy, McIlvain and Javois (1923) using dogs with Pavlov pouches and Thiry fistulae of the duodenum and jejunum, found that the application of 50 mgm. of histamine through the fistula to the mucosa was just sufficient to stimulate gastric secretion. In another paper (Ivy and McIlvain, 1923) it was reported that a solution of histamine containing 1 mgm. per cubic centimeter applied to the duodenal mucosa of dogs for from 20 to 30 minutes invariably produced, after a latent period of from 10 to 30 minutes, a definite increase in secretion of gastric juice. The increased rate of secretion persisted for from 1 to 2 hours. A dose of atropine sufficient to inhibit the gastric secretion elicited by a meal produced very little effect on the increased rate of secretion due to histamine.

Ivy and McIlvain found that the gastric response to a stimulus noted in Pavlov pouch dogs varied directly with the height of the basal secretion. In a continuation of this investigation Lim (1924) found that 1 mgm. of histamine given subcutaneously to one of these dogs produced an intense secretion lasting for about 1 hour. When the phase of increased secretion was over the juice had quite a different character from that of the basal secretion, in that the acidity was higher and the volume was less. When histamine was injected at hourly intervals a decreased effect on secretion was noted after the first hour but some response was obtained for as long as 11 hours. This diminution affected the secretion of fluid, while there was little or no reduction of HCl output. Lim, Ivy and McCarthy (1925) found that the application of histamine directly to the gastric mucosa in dogs stimulated gastric secretion.

Gutowski (1924) observed that histamine was absorbed by the venous route when it was injected subcutaneously in the posterior extremity of a dog where the only connection with the rest of the body was by way of the blood vessels. The characteristic effects were produced. Ligation of the thoracic duct did not interfere with the action of histamine administered subcutaneously.

In another paper Gutowski (1924) reported the results of a study of the rate of injection and absorption of histamine on gastric and pancreatic secretion. He found that when histamine was introduced slowly into a vein the amount of gastric juice produced was not appreciably less

than that elicited by the same amount of histamine administered subcutaneously. Very little gastric juice was produced by the rapid intravenous injection of histamine, but the pancreas was apparently stimulated more efficiently by the rapid than by the slow administration of the amine. Ivy and Javois (1924), in a more comprehensive study of the effect of rate of injection on the stimulation of gastric secretion by histamine, observed that when the amine was injected slowly intravenously at the rate of 0.0027 mgm. per kilogram per minute a definite stimulation of secretion in the Pavlov pouch of a dog was produced. They noticed that after the intravenous injection of histamine the subcutaneous administration of a dose, known to be adequate when given alone, caused little secretion. The diminution of the effect of a subcutaneous injection of histamine produced by a previous dose was thought to be greater when this preliminary injection was administered intravenously than when given by the subcutaneous route. Ivy and Javois noted that a very large dose of histamine given by stomach tube caused a definite stimulation of gastric juice in dogs equipped with Heidenhain pouches, which had been denervated for from 6 to 8 weeks. Ivy and Farrel (Ivy, 1925) have shown that the subcutaneous injection of histamine may produce secretion in a completely denervated gastric pouch which has been transplanted to the mammary gland of a dog.

Boyd (1927) found that the response of the Pavlov pouch of a dog to histamine injection was not appreciably affected by the previous oral administration of weak (2.5 per cent) sodium bicarbonate solution. When the strength of the solution was doubled and water was withheld secretion was depressed. When an abundance of water was available the stronger bicarbonate produced no effect.

Koskowski and Kubikowski (1929) have reported a parallelism between the gastric response elicited by injection of histamine to a dog and the power of defibrinated blood from the same dog to stimulate contraction of the isolated guinea-pig uterus. Confirmation of these results would be valuable.

Popielski (1929) has shown by gradually adapting frogs to mammalian temperature that under these conditions injection of histamine produces a more abundant flow of gastric juice than at the temperature natural for these animals. The flow of gastric juice was much more consistently elicited at the higher temperature.

Babkin (1930) has obtained evidence that the production of fluid, HCl and some other inorganic constituents by the gastric glands is affected by both nervous and humoral stimuli, while the secretion of

enzymes and other organic substances is governed by the parasympathetic nervous system. The digestive power of gastric juice was found to fall quickly under humoral stimulation. Babkin (1930) describes experiments of Vineberg who used a dog equipped with a Heidenhain pouch supplied with some parasympathetic fibres. One milligram of histamine given subcutaneously, followed in some cases by 7 mgm. of pilocarpine, was given. Histamine was found to produce a rise in the volume of the juice secreted, in the acidity and the concentration of chlorine. The peptic activity of the juice fell rapidly. After pilocarpine the peptic activity of the juice was greatly increased. Atropine did not affect the histamine secretion. Histamine is, therefore, a good stimulant of the acid producing cells but has much less effect on the peptic and mucoid cells of the dog's stomach. Gilman and Cowgill (1931) have recently reported results similar to those obtained by Vineberg and Babkin. Vineberg and Babkin (1931) suggest that a mixture of pilocarpine and histamine may prove to be a more useful stimulant of gastric secretion in human subjects than histamine alone.

Gastric secretion in man. Although previous workers had concluded from indirect evidence that histamine stimulates the gastric secretion in man, Carnot, Koskowski and Libert (1922) were the first to study this action directly. They noted that the volume of the secretion was increased as was also the total and free acid and digestive power of the juice. They attempted to eliminate psychic causes of secretion. The effect of histamine on gastric musculature was noted. Doses as large as 1.75 mgm. of the base administered subcutaneously were used. Matheson and Ammon (1923) used smaller doses than the previous workers and obtained increased secretion and no harmful systemic effects with 0.5 mgm. of the base. The maximum of peptic activity was reached in from 5 to 15 minutes and the total and free HCl maxima shortly after that. The rate of secretion attained its peak somewhat later. The results of these two investigations have stimulated a great deal of work and histamine injection is now widely used in testing the efficiency of gastric function. Although practically all the literature has been consulted, reference will be made to only a limited number of papers.

Lim, Matheson and Schlapp (1923) made observations on the change in the alveolar CO_2 of human cases after the subcutaneous administration of 0.5 mgm. histamine. When the juice secreted was immediately removed a rise of alveolar CO_2 followed in some cases by a fall was the usual but not invariable finding. The authors believed that their results are in accordance with the hypothesis that secretion of acid pro-

duces a rise of alveolar CO_2 and secretion of alkali a fall. They sometimes obtained the fall in alveolar CO_2 when the gastric secretion was immediately removed. This result suggests that histamine acts directly on pancreatic secretion although the authors conclude that the amine acts only indirectly on pancreatic or intestinal secretion. Later work has shown that the action of histamine on intestinal secretion is not entirely due to the stimulation of gastric secretion and its passage down the intestine. These investigators have related the histamine response with the basal level of gastric secretion. There appears to be a tendency for the optimum concentration of acid to be maintained. The volume of secretion and the output of acid are therefore related to the level of the basal outputs. They found that the acidity of the freshly secreted gastric juice was seldom as high as 0.5 gram per cent HCl. The increase in pepsin after histamine in patients was found to be irregular and could not be related to the acidity or volume of the juice. The administration of atropine subcutaneously or by stomach tube was ineffective in inhibiting the histamine response. These investigators (Lim, Matheson and Schlapp, 1923) have devised a technique for the conduction of the histamine test in which the secretions from stomach and duodenum are separately removed by continuous suction immediately after formation.

Dobson (1925) reported that histamine especially stimulated the secretion of acid in the gastric juice of patients. He suggested a classification of achylia on the basis of the response to a test meal, to histamine, or to neither. Ackman (1925) noted the production of a well marked "alkaline tide" when histamine was administered subcutaneously to patients with a normal or high gastric acidity. In cases of achlorhydria in which there was no response to histamine no "alkaline tide" developed. The fact that there is no change in the pH of the urine in these cases of achlorhydria is taken to indicate that the "alkaline tide" which is found in normal cases is not due to increased metabolic activity produced by histamine. Gompertz and Vorhaus (1925), Gompertz and Cohen (1929), Carnot and Libert (1925) and many others emphasize the safety of small doses of histamine and the superiority of the histamine test over the test meal. Bockus and Bank (1927) found that only approximately half the cases diagnosed as achlorhydria after the Rehfuss test failed to secrete acid juice after histamine. Repeated fractional tests in about 40 per cent of the originally negative cases, however, gave positive results. In case of hyperacidity no response to histamine was observed. In this and in other reports the fact that his-

tamine stimulation is not an established physiological method of exciting gastric secretion is stressed. Henning (1928) emphasized the necessity of conducting several histamine tests before concluding that the gastric cells have completely lost the power to secrete. There is fairly general agreement that atropine does not prevent the action of histamine on the human gastric glands (Delhougne, 1926; Bickel, 1927). Delhougne noticed a lessened and slowed reaction to histamine in mildly tuberculous patients. In severe cases the abnormality of secretion was more particularly related to the pepsin content of the juice.

Rachon and Walawski (1928) studied the effects of repeated subcutaneous administrations of 0.5 mgm. histamine to a patient with a gastric fistula. No appreciable change of blood pressure was noted, while the secretion of juice, after a latent period of from 10 to 15 minutes, was increased for about 1½ hours after each injection. Katsch and Kalk (1926), Faber and Holst (1928), and Johansen (1929) administered histamine to patients with achylia associated with pernicious anemia and other conditions. Faber and Holst reported an increase in the pepsin content of the juice in many of their cases. There is fairly general agreement that there is no definite relationship between the increases in acid and proteolytic power produced by histamine. Mogena (1927) reported that the intravenous injection of histamine in man did not stimulate gastric secretion. A slow intravenous injection would undoubtedly give positive results. He reported the results of the use of histamine, subcutaneously administered, in various clinical conditions.

Very large doses of histamine, 200 mgm., administered by stomach tube to a man (Ivy, McIlvain and Javois, 1923) produced definite gastric secretion. No toxic symptoms were observed. Other investigators have reported both negative and positive results with smaller doses.

Molinari-Tosatti (1929) has compared the effect of histamine and a test meal on gastric secretion in man. Secretion lasted longer after the meal than after histamine. The histamine secretion had a higher free HCl in the early stages but the peptic activity was much less. Variable response of the peptic activity to histamine has been noted by numerous investigators.

Grimbert and Fleury (1929) give the following average values as results of the analysis of human gastric juice stimulated by histamine. The dry residue from a litre of juice weighed 6.37 grams. About 62 per cent was organic matter and 38 per cent inorganic. The inorganic fraction contained about 43 per cent Cl. K, Na, PO_4, Ca and traces of Mg were also found. Berglund, Wahlquist, and Sherwood (1926)

believed that histamine gastric juice contained little if any neutral chloride, but several other investigators found that the increase in chlorine was not completely dependent on acid secretion (Mogena and Fernandez, 1928).

Polland (1930) observed that the administration of atropine to patients caused a marked decrease in the volume, concentration and total output of pepsin of the gastric juice produced by injection of histamine. The total output of acid was apparently only slightly reduced by atropine. Libert and Harmelin (1926) found that histamine stimulated the gastric juice in infants. Neale and Klumpp (1930) have studied the secretion of gastric juice elicited by histamine in children of various ages.

The local effects of histamine administered subcutaneously may be counteracted by mixing a small amount of epinephrine with the histamine. It is stated by various workers that this amount of epinephrine does not seriously interfere with the gastric response, but more exact evidence on this point is desirable.

It has been suggested that the stimulating effect of coffee on gastric secretion may be partly due to histamine or related substances (see Distribution). This suggestion is not tenable however, since there is no satisfactory evidence that histamine or histamine-like substances in food or in the products of gastric digestion act as a stimulant of gastric secretion. It apparently requires relatively large amounts of histamine to produce effects when the active material is administered enterally, although Fényes (1930) has reported definite effects from 2 to 12 mgm.

Kalk (1929) has obtained very interesting results by a study of gastric secretion in a case of dermographia. Mechanical irritation of the skin was followed by an increased flow of gastric juice.

Pancreatic secretion. Very little information is available on the action of histamine on pancreatic secretion. Dalé and Laidlaw (1910) noticed that the intravenous administration of the amine produced a flow of pancreatic juice in anesthetized dogs. This action was abolished by atropine. They state that the action of histamine on pancreatic secretion is of a different type and of a much lower order than that of secretin. There has, however, been some controversy over the significance of the vasodilator principle present in many secretin preparations, but it is now established by the work of Mellanby and Ivy, that histamine is not a constituent of the purest secretin preparations. In 1925 Parsons studied the histamine content of various secretin preparations using the Koessler and Hanke quantitative Pauly reaction method. She found

that the amount of histamine contained in the preparations was not significant physiologically. Histamine is a much more powerful vasodilator and a much less potent secretagogue than secretin.

Lim and Schlapp (1923) believed that histamine stimulated pancreatic secretion only indirectly as a result of the entrance of gastric juice into the duodenum. Osborne (1928) and Neale and Klumpp (1930) have supported this conclusion. The positive results of Molinari-Tosatti (1928) who obtained pancreatic secretion following histamine injection in an animal in which the pylorus and the common bile duct were completely closed, furnish strong corroborative evidence that histamine exerts a direct action. Gutowski (1924) found that the rapid intravenous injection of histamine produced a good flow of pancreatic juice while much less was elicited by the slow intravenous or subcutaneous administration of the amine. MacKay and Baxter (1931) have reported that histamine when applied in 2 per cent HCl to the duodenum produces a flow of pancreatic juice in animals in which the pylorus and common bile duct are ligated. The mechanism by which the effect is produced is not as yet known.

Glands of the small and large intestine. Koskowski (1926) was able to show that the subcutaneous administration of histamine to dogs stimulated, with a very short latent period, a secretion of the glands in the small intestine. The secretion which is prevented by atropine, begins within two minutes after the subcutaneous injection of histamine. Nechoroschew (1929) added the observation that histamine administered subcutaneously or by duodenal fistula increases the flow of intestinal juice. Koskowski (1926) has also reported a small secretion of the glands of the large intestine after the subcutaneous administration of histamine to dogs.

To summarize this section—it is established that histamine administered intravenously, subcutaneously or intramuscularly, stimulates the salivary glands in dogs and cats. The action on the different salivary glands has not been thoroughly studied. The effect of histamine which produces vasodilatation and increased blood flow through the glands, is reduced by marked lowering of the systemic blood pressure (Fröhlich and Pick, 1913), but MacKay finds that the blood flow through the gland is not materially lessened by a moderate lowering of blood pressure. Popielski's demonstration (1920) that an anemia of the central nervous system may produce a reflex stimulation of the salivary glands, which is not demonstrable after cutting the chorda tympani, is interesting in this connection. Atropine strongly inhibits the histamine stimulation

of salivary glands. Pilocarpine may produce a reversal of the action of histamine on the blood flow through the glands. Stimulation of the chorda tympani before histamine injection augments the effects of the amine. The demonstration that nerve stimulation may produce a histamine-like substance in other parts of the body leads one to suspect that stimulation of the chorda may exert its effects by releasing a vasodilator substance locally. The glands are not made more sensitive to histamine by one injection of the amine. There is evidence that there is also an unexplained mechanical effect of histamine on salivary secretion. Some effect of histamine is still demonstrable after administration of ergotoxin and atropine.

It is established that histamine administered subcutaneously, intramuscularly or slowly intravenously in doses of less than 1 mgm. stimulates gastric secretion in laboratory animals and man. Moderate or large doses given by mouth or applied to the stomach and various parts of the intestine are also effective. Local vasodilatation probably facilitates the action of the glands but there may be a more specific action. Doses of histamine which do not produce any perceptible lowering of blood pressure have been reported to produce secretion, but the minimum depressor dose is practically the same as the smallest amount which produces an increase in gastric secretion. When the blood pressure is profoundly lowered by intravenous administration of histamine there is no gastric secretion. Atropine has little or no effect on the production of gastric juice by histamine. Histamine stimulates the cells in a denervated gastric pouch. Although there is an increase in the peptic activity after histamine the enzyme producing cells are not stimulated as vigorously as those which form HCl. The enzyme response is variable in the same individual. The gastric secretory mechanism affected by histamine is not easily completely fatigued, but the effect of an injection of the amine is greatly reduced by the recent previous administration of the substance.

There is no satisfactory evidence that histamine produces beneficial effects in patients with diminished digestive functions, although its use has been advocated in these conditions. Many human individuals in whom achylia would be diagnosed on the findings obtained after a test meal, secrete acid in response to histamine. When both gastric and duodenal secretions are removed immediately after formation, subcutaneous histamine injection (0.25–0.5 mgm.) provides a safe and convenient method of studying the ability of the gastric glands to form acid in response to a standardized stimulus. When other aspects of gastric

function are to be studied test meals or other stimulating agents are preferable. In a variety of clinical conditions there may be a temporary loss of the power of the gastric glands to produce acid. One negative histamine test is, therefore, not conclusive evidence of achlorhydria.

Histamine may exert a direct action on the pancreas, and on the glands of the small and large intestine of animals. These effects are not demonstrable after atropine. The action of the amine on the pancreas in man is not easy to investigate, but the available facts suggest that the small doses used exert little or no direct effect.

Other glands. Histamine stimulates the secretion of tears in animals (Dale and Laidlaw, 1910) and in man (Gompertz and Cohen, 1929). Dale and Laidlaw also report that histamine produces a secretion of bronchial mucus. Various other glands have been investigated but no effect of histamine has been observed.

THE FALL IN BLOOD PRESSURE PRODUCED BY HISTAMINE. *Effect on the heart.* Dale and Laidlaw (1910) observed that small doses of histamine increased the force and frequency of the heart beat of rabbits or cats. The effects on the organ *in situ* and when isolated from the body were studied. Using larger doses a weakening of the heart was noticed. Führer and Starling (1913) reported that a rise in pulmonary arterial pressure was produced when a large dose of histamine was added to the blood in a heart-lung preparation of a dog. The heart volume and the pressure in the left auricle after a transient decrease exhibited an increase. The heart, however, soon failed and dilated. The effects of small doses of histamine were not studied. Rühl (1929) has reported that the left ventricle of cats is weakened by histamine. In the heart-lung preparation of the dog he found that, under exacting conditions, histamine produced a lasting injury to the heart. Irregularities of the heart beat with extrasystoles were observed. In the isolated heart most observers have found that small doses increase the rate and strength of the beat (Einis, 1913; Rothlin, 1920; Abe, 1920; Gunn, 1926). Oppenheimer (1929) has compared the effects of certain heart extracts and of histamine on the isolated perfused heart of the rabbit. The results were very similar. An increased amplitude and in many cases an acceleration of the rate of the heart beat was noted. Subsequently diminution of the rate and failure of the heart were observed. These and other results make it very unlikely that a specific "heart hormone" has been isolated from cardiac muscle. The reports of Demoor and Rylant (1925) and Haberlandt (1924) should be consulted for further information on this subject. When the amount of histamine

is increased the heart is weakened and heart block may be observed. Hashimoto (1925) showed that the intravenous administration of large doses of histamine to dogs caused a delay in auriculo-ventricular conduction and a temporary partial or complete heart block. The delayed conduction lasted for about twelve minutes, and the heart block about one-third as long. Schenk (1922) in an electrocardiographic study detected no effect of histamine on the heart of the rabbit, but in man he noticed ventricular extrasystoles and arrhythmia after a large dose. Dixon and Hoyle (1930) have observed increased cardiac output in the cat with small doses of histamine while large doses produced diminution, and when the systemic blood pressure had fallen considerably the heart dilated. These investigators believe that injections of moderate amounts of histamine always weaken the heart of the dog.

Effect on coronary circulation. Dale and Laidlaw (1911) found a decrease in coronary outflow in the perfused rabbit heart after the addition of histamine to the perfusion fluid. Barbour (1913) demonstrated that histamine caused a contraction of isolated rings from the coronary artery of the ox. Gunn (1926) and Cruickshank and Subba Rau (1927) found that histamine produced a dilatation of the coronary vessels in the cat's heart. The latter investigators found that histamine caused constriction of rings from the isolated coronary arteries of dog, ox, and man. In perfusion experiments using pieces of coronary artery from the rabbit's heart histamine regularly produced a diminution of flow. Anrep (1926) stated that histamine in a dilution of 1:5,000,000 contracted the isolated coronary rings of the ox and dog. He also reported that Lihacheva observed a constriction of the coronary vessels of the rabbit's heart after histamine. Sumbal (1924) found that the coronary arteries of the tortoise were consistently dilated by histamine either applied locally to the superficial coronary arteries or added to Ringer's solution used as perfusion fluid. Atropinization did not affect this action of histamine.

Rühl (1929) has demonstrated a definite increase in the coronary outflow of the dog's heart in the heart-lung preparation after the injection of histamine. In the opinion of Feldberg and Schilf (1930) this increase is not only due to relaxation of the cardiac muscle but also to independent coronary dilatation, since small doses which produced no perceptible change in rate or minute volume still increased the coronary outflow.

The evidence suggests that small doses of histamine have little if any effect on the heart. Somewhat larger amounts increase the force and rate of the beat in most species. The effect of very large doses varies in

the different species and is in part attributable to the diminution in venous inflow through the action on the portal, pulmonary, or systemic vessels and in part to a weakening of the myocardium by a direct action or by producing, in some species, constriction of the coronary arteries. It is established, however, that even after large doses of histamine the cat's heart may beat very forcibly and effectively if adequate venous inflow is provided.

The action on the blood vessels. Ackerman and Kutscher (1910) and Dale and Laidlaw (1910) demonstrated that intravenous injections of histamine produce profound lowering of the blood pressure of the anesthetized cat. The latter investigators studied also the dog, monkey, and fowl. In the cat and dog they found that the fall in pressure could frequently be divided into two phases. The first phase was transient and was attributed to constriction of the pulmonary vessels and a reduced venous inflow to the heart. The duration of the second phase increased with the dose, and the fall in blood pressure was shown by plethysmographic observations to be accomplished by peripheral vasodilatation, which in later experiments was shown to be a primary action of the amine and independent of the integrity of the sympathetic nervous system (Dale and Laidlaw, 1911) and of the cerebrospinal nerves (Dale and Richards, 1918). Burn (1922) demonstrated that when the nerves to a limb of a cat are cut and sufficient time is allowed for complete degeneration to occur, the usual increase in volume after small doses of histamine is not obtained. Lack of the sympathetic fibres alone did not produce this result. Burn believes that loss of the posterior root fibres results in a decrease of capillary tone and that under these conditions the volume of the limbs decreases passively as the systemic arterial blood pressure falls. As Krogh (1929) has pointed out, any considerable capillary dilatation would have been obvious, and as this was not observed it appears that loss of nerve supply affects the sensitivity of the capillaries to histamine in a manner which is not completely understood.

Dale and Laidlaw (1910) found that histamine produced vasoconstriction in isolated perfused tissues of the cat while in the intact animal vasodilatation in the same tissue was observed. This paradoxical effect of histamine on the vessels of perfused tissues has stimulated a great deal of work. Mautner and Pick (1915) demonstrated that the hepatic vessels were constricted in the perfused dog liver by histamine. This result, which is discussed under another heading, led these investigators to suggest that hepatic obstruction was the main feature in the produc-

tion of the fall of blood pressure. Dale and Richards (1918) demonstrated that the latent period of histamine action was shortest after intra-arterial injection, somewhat longer after peripheral intravenous injection, and longest after introduction into the portal vein. These results have been confirmed by later workers. Furthermore, in the eviscerated cat, in which the circulation to the liver is practically eliminated, histamine still lowered blood pressure. These results demonstrated that there is one phase of histamine action which is independent of change in hepatic circulation, and in which the pulmonary circulation is not playing a major rôle. In experiments using a cat mesentery preparation perfused with hirudinized blood histamine decreased the blood flow. Since the blood vessels of this preparation are arteries and arterioles histamine constricts these parts of the vascular tree, under these conditions in this species. Dale and Richards noted that application of histamine to the surface of the pancreas produced a reddening and later an edema. They also showed that the injection of histamine caused a flushing of the pad of the denervated foot of the cat. Before the injection of histamine the denervated foot was pale but the blood flow was increased. The arterioles were dilated and the capillaries constricted. The flushing indicates capillary dilatation. These results are very significant in connection with the phenomena of independent capillary movement as well as with the location of histamine action. Dale and Richards also investigated further the "histamine paradox" and found that under the conditions of their perfusion experiments either red blood cells or epinephrine was necessary to demonstrate the vasodilator effect of histamine. More recently Burn and Dale (1926) using an improved perfusion apparatus have been able to obtain increased tissue volume and increased venous outflow after histamine in the absence of red blood cells or added epinephrine. The explanation of this success apparently was that the capillaries regained tone and maintained it long enough to permit the demonstration of its relaxation by histamine. The period of maintenance of the regained tone varied in the different species (cat, dog, and monkey). In the cat a moderate or large dose of histamine accelerated its disappearance. The capillary tone can be maintained for long periods by the infusion of small amounts of epinephrine. The action of the red blood cells in the earlier experiments may have been in either or both of two ways—by increasing the oxygen carriage of the perfusion fluid, and thus presumably the tone of the capillaries, or by interfering with the passage of the blood through the capillaries, and in this way assisting the partially relaxed vessels to

contribute to the peripheral resistance. Hemingway and McDowall (1926) and Atzler and Lehmann (1927) have emphasized the relation between accumulation of acid products and the loss of capillary tone. The former investigators have shown that histamine vasodilatation may be demonstrated for relatively long periods in isolated perfused tissues if alkaline fluid is infused before the death of the animals or if alkaline perfusion fluid is used. When the red blood cells are present the better oxygen supply to the capillaries will assist in preventing the accumulation of acid metabolites. The "histamine paradox" is therefore explained and is attributable to the loss of capillary tone which takes place to a greater or less extent in perfusion experiments.

McDowall (1923) showed that small doses of histamine produced a rise in venous pressure in cats under light anesthesia. When the anesthesia was deepened this effect of histamine was not observed. It is probably significant that constriction of the pulmonary arterioles by small doses of histamine is also abolished by deep anesthesia. In unpublished work McDowall has found that when the rise in pressure in the pulmonary artery is prevented by means of a valve, the rise in venous pressure produced by histamine is greatly reduced and occurs during recovery of the blood pressure. McDowall admits that capillary dilatation is an important factor in the decrease in blood pressure produced by small doses of histamine, but he emphasizes the possibility that constriction of the pulmonary arterioles may be a recognizable component of the effect of small as well as of larger doses of the amine. McDowall and Worsnop (1924) have obtained evidence that the effects of small doses of histamine are not so evanescent as the transient decrease in arterial blood pressure suggests. The action of the amine is, in a sense, cumulative since an experimental animal is less capable of recovery from a second dose than from the first, although the arterial blood pressure returns to normal soon after the first injection. The fact that, after a small dose of histamine, an increased response to acetylcholine, an increased velocity of pulse wave, and, in some cases, a return of the volume of a limb to less than the basal level may be observed, suggests that the capillaries may remain partially dilated and the prompt recovery of the arterial blood pressure be effected, in part, by arterial constriction. These investigators also stated that a succession of doses of histamine from which the arterial pressure recovered, produced a progressive fall of venous pressure.

Inchley (1923) noted that histamine constricts the mesenteric veins of the cat, and in a later paper (Inchley, 1926) he has supported the hypo-

thesis that the profound fall in blood pressure produced by large doses of histamine is produced by venous constriction and passive dilatation of the capillaries. There is no doubt that venous constriction in certain organs must be considered in the mechanism of the production of histamine shock in some species, but it is profitable to consider at this point whether general venous constriction is an essential preliminary factor in the production of the fall of blood pressure observed after the administration of small doses of the amine, i.e., is there direct capillary dilatation without preliminary venous constriction? Or is there always an increased venous resistance before the capillaries dilate? In perfusion experiments Inchley has shown that histamine in certain doses may constrict the veins without affecting the arteries. One difficulty in the acceptance of Inchley's theory is that Dale and Richards consistently found an increased limb volume and increased venous outflow in perfusion experiments after the addition of histamine. Inchley has, however, been able to produce mechanically a venous obstruction which, after a latent period, results in an increased outflow. The latent period is considerably longer than that observed by Dale and Richards. The conditions of Inchley's perfusion experiments, in which the capillaries were present, were such that capillary tone would not be well maintained and capillary dilatation, therefore, would not be observed. These considerations make it unlikely that venous constriction is an important effect of small doses of histamine, and the results of direct observations furnish satisfactory evidence that capillary dilatation can be produced by small doses of histamine, without preliminary venous constriction.

Effect on the arterioles. Dale and Richards (1918) found that the preparation made from the cat mesentery, the vessels of which are arterial, showed constriction after the addition of histamine to the perfusion fluid. In a similar preparation from the dog, Burn and Dale (1926) observed that histamine produced some dilatation. Bauer and Richards (1928) confirmed Burn and Dale's results and reported that the isolated iliac artery of the dog exhibits constriction after histamine. Other observers have also noted that histamine constricts the isolated arteries in various species (Barbour, 1913; Rothlin, 1920, and Katz, 1929). Ranson, Faubion and Ross (1923) have reported that histamine injected intra-arterially into the leg of a dog produces a transient dilatation of the vessels. The increased pulsation indicated that arterioles as well as capillaries were involved. Burn and Dale (1926) obtained evidence that histamine produced dilatation of the arteries of the perfused limb of the dog or monkey. Harmer and Harris (1926) reported

that histamine caused dilatation of the skin arterioles in human individuals. Carrier (1922) also reported that the human arterioles were dilated by histamine. The action of histamine on the cerebral, coronary, pulmonary and renal vessels is considered under other headings.

Dale in 1929 and in earlier papers, has drawn attention to the shift in the locus of action of histamine as the biological tree is ascended. In rodents arterial constriction may be the predominant effect. In the cat there is a slight constriction of the arterioles and dilatation of the capillaries. In the dog and monkey both arterioles and capillaries dilate. In man also the arterioles and the capillaries of the skin and of the brain dilate.

Effect on capillaries. Hooker (1921) made direct observations on the effect of histamine on the capillaries and venules of anesthetized cats. He found that within a few minutes after the injection of large doses of histamine both types of vessels were dilated and filled with stagnant blood. In a few cases Hooker observed a transient preliminary constriction of both capillaries and venules. In one experiment he noted a decrease in venous pressure accompanying the arterial fall. Rich (1921) using the omentum of the etherized cat found that injection of histamine exerted a local vasodilator effect upon capillaries, small arterioles and venules. New capillaries were observed to open up under the influence of histamine. In histamine shock a very pronounced dilatation of these vessels was found. Rich observed similar changes in the small vessels after the local application of histamine, and he studied the vascular changes produced by histamine by fixing and staining sections of the omentum as well as by observations in the living animal.

Carrier (1922) demonstrated that histamine in a dilution of 1:1000, 1:5000, 1:10,000 applied to the base of the finger nail in normal human beings produces a widening of the capillaries and an acceleration of capillary blood flow. These observations were made somewhat difficult by the fact that the capillaries in the area investigated were well filled before the application of histamine, and also by the production of edema. Florey and Carleton (1926), observing the mesenteric capillaries under saline in the anesthetized cat, found that the injection of histamine into the saphenous vein produced a dilatation of the capillaries and an opening up of many which had not been apparent. The venules were dilated. When a piece of the mesentery was removed a few minutes after the injection of histamine a pronounced constriction of the veins was noted. This phenomenon had been observed previously by Dale. When the capillaries of the omentum were fully dilated by histamine, application

of "pituitrin" caused definite constriction. Hartman, Evans and Walker (1929) studying the capillary circulation in the sartorius muscle of anesthetized or decerebrate cats, found that both small and large doses produced dilatation of the capillaries and venules. With the larger amounts constriction of the arterioles, arteries and veins was noted.

Capillary dilatation which brings many new capillaries into view can be observed in the ear of the rabbit after injection or application of histamine. The central artery of the ear is usually strongly contracted (Feldberg, 1927; Flatow, 1929).

Veins. Franklin (1925) found that histamine produced a contraction of isolated rings from various large veins of the sheep. Inchley (1926) observed contraction of veins in the pig and cat. The effects on the hepatic, cerebral and other veins are referred to under other headings and need not be discussed here. The small veins of the human skin dilate after local application of histamine, while the larger ones may exhibit a slight constriction.

Effect on the renal circulation. Dale and Laidlaw (1910) noticed that the volume of the kidney decreased during the fall in blood pressure produced by histamine. The extent of the decrease suggested an active constriction of the renal vessels and not merely a passive effect accompanying the fall in blood pressure. Manwaring, Monaco and Marino (1922) noticed an increase in the resistance to a fluid perfused through the isolated kidney of the dog when histamine was added. Dicker (1928) perfused the isolated kidneys of the dog with Locke's solution containing histamine and found a decreased venous outflow. Morimoto (1928) has studied the blood flow through the kidneys before and after injection of small amounts of histamine into the renal artery. He believes that histamine constricts the renal vessels. Histamine constricts the isolated renal artery of the horse, ox, and pig (Rothlin, 1920). Ganter and Schretzenmayr (1929) believe, however, that in various concentrations histamine has a dilating effect on the capillaries of the intact kidney of the cat. They employed a method previously elaborated by Ganter. Paralysis of the thoracic sympathetic or of the bulbo-sacral nervous systems apparently did not prevent this action of histamine. These results are difficult to correlate with the decreased volume and increased resistance to perfusion produced by histamine. It may be that the constriction of the arterioles is the predominant effect under certain conditions, while under others it is capillary dilatation.

Effect on the hepatic circulation. This phase of the action of histamine has been studied in liver perfusion experiments and in the intact animal.

The difference in the effect of histamine on the liver of various species is very marked. Very definite effects are produced in the dog but the amine has little action on the liver vessels of the other animals which have been investigated. Mautner and Pick (1915, 1929), have demonstrated that histamine contracts the hepatic veins in the dog liver in perfusion experiments or in intact animals. The effect of moderate doses is very striking. Blood is retained in the capillary spaces of the liver and later, in the intact animal, the viscera drained by the portal vein may share in the congestion. In perfusion experiments using Ringer's solution with 10 per cent defibrinated blood Baer and Rössler (1926) reversed the direction of the perfusion, i.e., fluid was introduced into the hepatic veins and emerged from the portal. Histamine can be shown under these conditions to produce an obstruction to the inflow of the perfusion fluid. Manwaring, Monaco and Marino (1922) found that the liver perfused with Locke's solution was not as sensitive to histamine as other observers have demonstrated it to be when blood is used. Feldberg, Schilf and Zernik (1928) have studied the portal vein pressure after the intravenous injection of histamine in dogs. Their tracings demonstrate a profound fall of arterial pressure, a slight fall of systemic venous pressure, and a very definite rise of portal vein pressure. When the injection was made into the portal vein the rise of portal vein pressure was much greater than after intrajugular administrations. With very small doses the portal vein pressure fell with the systemic venous pressure. Bauer, Dale and Richards (Dale, 1929) have perfused dog liver through the portal vein and hepatic artery. The addition of a small amount of histamine produced an obstruction to the outflow and a swelling of the liver. This effect, which was attributed to constriction of the hepatic veins, was relieved by the addition of epinephrine. Dale (1929) makes the interesting suggestion that the histamine which is present in the intestine may be absorbed during digestion and that this may cause a transient retention of the blood in the liver. Larger amounts of histamine may produce profound capillary engorgement and loss of fluid into the tissue spaces. In muscular exercise the liberation of small amounts of epinephrine may make available (Bauer, Dale and Richards, 1929) blood which has been stored in the liver. It should be noted that hepatic constriction is not the only mechanism by which histamine produces a fall in blood pressure in the dog. In Eck-fistula animals histamine is still effective (Mautner and Pick, 1929) and in perfusion experiments and in the intact animal peripheral dilatation of arterioles and capillaries can be easily demonstrated.

Hunt (1917), Mautner and Pick (1929), Bauer, Dale and Richards (1929) and Ganter and Schretzenmayr (1929, 1930), found that there was little or no constriction of the hepatic veins in the cat after histamine. Feldberg (1929) obtained variable effects. In some cases a slight constriction was noted. With large doses Dixon and Hoyle (1930) demonstrated a definite rise in portal vein pressure in cats, but the amount was insignificant compared to that produced by the same dose in dogs. Dale and Laidlaw (1919) observed no participation of the cat liver in histamine shock.

In the guinea pig, rabbit and monkey, histamine has little or no effect on the hepatic veins (Mautner and Pick, 1915, 1929; Feldberg and Schilf, 1930; Bauer, Dale and Richards, 1929; Dixon and Hoyle, 1930).

The effect of histamine on the hepatic veins is therefore largely restricted, as far as we know at present, to the dog. The major rôle played by the liver in anaphylactic shock in this animal illustrates the great similarity between the effects of histamine and anaphylaxis in the various species. This variation in the action of histamine on the liver in the different species is in part attributable to the different amounts of smooth muscle about the hepatic veins. In the dog Arey and Simons (1920) found that these vessels are equipped with a stout covering of smooth muscle, which must be particularly sensitive to histamine.

Effect on the pulmonary circulation. In the pulmonary circulation again the difference in histamine action in various species is very great. It is apparently established that bronchial constriction, which is an outstanding effect of histamine in certain species, does not interfere with the passage of the blood.

Dale and Laidlaw (1910) demonstrated that large doses of histamine cause a constriction of the pulmonary artery in the cat. In the intact animal a rise in pulmonary artery pressure was noted, and in lung perfusion experiments a decreased venous outflow. The experiments of Dale and Richards (1918) and of McDowall (1923) which are important in this connection, have been discussed.

Contractions of isolated rings of the pulmonary artery of the rabbit (Barbour, 1913), horse, cattle (Rothlin, 1920), and swine (Rothlin, 1920; Inchley, 1926) after the application of histamine have been observed.

The controversy between Anderes and Cloetta (1914, 1916) who believed the pulmonary vessels of the cat to be constricted by histamine and the vascular volume of the lung to be decreased, and Weber (1912, 1914), who was convinced that the vessels are dilated, will not be reviewed in detail. It is established that the pulmonary arterial pressure rises (Dale

and Laidlaw, 1910; Anderes and Cloetta, 1916; Rühl, 1929; Dixon and Hoyle, 1930). Dixon and Hoyle failed to find any evidence that the rise of pressure was due to constriction of the pulmonary arterioles. In their experiments the outflow from the pulmonary veins was increased. The rise in arterial pressure and the increased venous outflow were attributed to increased blood supply from the right heart. As has been discussed above, small doses of histamine dilate the coronary arteries and increase the rate and force of the heart in the cat. With larger doses Dixon and Hoyle believe that the left heart is weakened before the right and that the blood is shunted through the coronary arteries from the systemic to the pulmonary circulation. It is significant that these observers have never noted any signs of pulmonary edema.

Since histamine causes little contraction of the hepatic veins of the cat, insufficient venous return is not a significant factor in changes in pulmonary pressure. Dixon and Hoyle, however, find an increased portal vein pressure after large doses of histamine.

In the dog the constriction of the hepatic veins by histamine will reduce the volume of the blood available for the right heart, and this offers a reasonable explanation for the main effect of histamine on the pulmonary circulation in this species, i.e., fall of pulmonary arterial pressure, decreased blood flow through the lungs, and decreased lung volume. Constriction of the coronary vessels and a direct myocardial effect may contribute to these results.

The preliminary effects of histamine on the pulmonary circulation of the dog, that is, increased pulmonary arterial and pulmonary vein pressures, increased lung volume and slight increase in right auricular pressure, are transient and are probably attributable to increased cardiac output and slight constriction of the pulmonary arterioles (Dixon and Hoyle, 1930).

In the rabbit the effect on blood pressure, as discussed elsewhere, is variable. When the systemic arterial pressure falls there is no constriction of the hepatic veins. The pressure in the pulmonary artery rises and the outflow from the pulmonary veins decreases. The coronary arteries in the rabbit's heart are constricted by histamine and this may assist in producing the failure of the heart after large doses (Dixon and Hoyle 1930).

Effect on the cerebral circulation and the pressure of the spinal fluid. Lee (1925) studied the reactions of the vessels in the pia mater of the etherized dog after the intravenous injection of histamine. No significant change in the diameter of the vessels was noted. A decrease in

the size of the brain was observed. Direct application of histamine to the brain was not studied. Histological studies of fixed specimens failed to reveal any effects of histamine. Lee has measured the pressure of the cerebrospinal fluid in dogs and cats and has correlated the changes in pressure produced by histamine with the variations in arterial and venous pressures. With a dose of 0.05 mgm. of histamine the fall in cerebrospinal fluid pressure of a cat weighing 2650 grams was approximately 40 mm. of Ringer's solution. The rise in systemic venous pressure was practically the same. Lee has emphasized the danger of assuming that all the capillaries of an animal react in the same way to a given stimulus. Numerous observers have reported flushing of the face and a throbbing headache after the injection of histamine in man (Jaeger, 1913; Schenk, 1921; Harmer and Harris, 1926; Fényes, 1930). Weiss and Lennox (see Forbes et al.) have observed a rise in cerebrospinal fluid pressure after the intravenous administration of small doses of histamine in the unanesthetized human subject. The amount of histamine was not sufficient to cause a fall of arterial pressure. Weiss (see Forbes et al.) has administered a small dose of the histamine intravenously during a cranial operation to a patient under luminal and paraldehyde narcosis. A definite increase in the volume of the brain resulted. The cortex bulged outward and increased pulsation and flushing of the brain surface were noted. Forbes, Wolff and Cobb (1929) have studied the effects of histamine hydrochloride applied to the surface of the brain and injected intravenously and intra-arterially into cats under isoamylethyl barbituric acid anesthesia. In twenty-one experiments the pial vessels (small arteries and capillaries) in every case except one, showed definite dilatation. The spinal fluid pressure always rose after intravenous injection of histamine. In the one case in which the vessels under observation did not dilate after intravenous injection, histamine had been previously applied locally and the vessels were probably maximally dilated. Local application of histamine to the brains of these animals always produced vasodilatation. The animals studied by Lee were etherized, and Forbes, Wolff and Cobb have found that under these conditions the vessels were maximally dilated, and for this reason histamine produces either no further effect or a vasoconstriction may be observed. It is suggested by these authors that the greater hemorrhage from the bone and meninges under ether compared with "amytal" anesthesia may be of practical significance. Using ether, a fall in spinal fluid pressure was obtained after histamine. In animals under "amytal" intravenous histamine caused dilatation of the great pial ar-

tery, while the systemic arterial pressure fell. To demonstrate that the dilatation of the cerebral vessels produced by histamine was not a result of the rise in venous pressure, the pressure in the systemic veins was raised by intravenous injections without producing any dilatation of the vessels of the pia mater.

These interesting investigations, in addition to what they teach about the action of histamine and the physiology of the cerebral blood vessels, illustrate again the profound effects of anesthesia upon the physiological action of many chemical substances.

LYMPH FLOW. Dale and Laidlaw (1911) observed an increase in lymph flow from the thoracic duct of dogs anesthetized with an A.C.E. mixture, when a dose of 2 mgm. of histamine was given intravenously. The lymph was slightly more concentrated than the normal, and clotted readily. Although other brief references to increased lymph flow after histamine have been encountered in the literature, the results of a comprehensive investigation of this action of histamine have not been published.

EFFECTS ON THE COMPOSITION OF THE BLOOD. Many attempts have been made to correlate effects produced by histamine with the conditions observed in traumatic shock, in anaphylactic shock, and in acute intestinal obstruction. The changes which take place in the blood in these three conditions have frequently been compared with those produced by histamine.

In their paper on histamine shock, Dale and Laidlaw (1919) laid the foundation for an explanation of many of the changes in composition of the blood subsequently observed by others. In histamine shock in anesthetized dogs and cats there is a marked increase in the red blood cell count. This was considered to be due either to a loss of fluid from the blood into the tissues, or to a release of corpuscles from some reservoir into the blood. Direct measurements of the plasma volume demonstrated that the first explanation is the correct one. For example, in one experiment there was a loss of plasma volume amounting to 41 per cent. At the same time there was an increase of 40 per cent in the hemoglobin value. These results were attributed to increased permeability of the capillary wall which permitted the passage of plasma into the tissues. In histamine shock there is also a diminution of leucocytes, and an increase in blood platelets. The increase in blood concentration is most important in view of later findings.

Underhill and Roth (1922) have investigated the blood concentration in rabbits after injections of histamine. They found that relatively

large amounts given intravenously produced either no change or a decrease which was insignificant. This result is quite different from that found in cats or dogs. The effects of histamine on the rabbit have been discussed elsewhere.

In an investigation of the effects of extirpation of the adrenals in cats, Kellaway and Cowell (1922) found that histamine produced an increase in blood concentration greater than that observed in normal animals, when one adrenal had been removed. The increase was greater still after the removal of both glands.

In 1911 Dale and Laidlaw observed a decrease in leucocytes in the blood following the administration of histamine. Bloom (1922) advanced the suggestion that an increased migration of leucocytes into the tissues in inflammations might be due to histamine, but he was unable to support this very interesting hypothesis by experimental results. Wolf (1921) studied the effects of a number of agents on the bloods of various species *in vitro*. She found that histamine had a pronounced chemotactic effect on the leucocytes of man and dog but less effect was observed on rabbit leucocytes. On the other hand Grant and Wood (1928) were unable to demonstrate that histamine had any effect on the migration of leucocytes in the frog, rabbit or in human subjects. Working with rabbits, Findlay (1928) found that after intradermal inoculations of histamine there was a local accumulation of leucocytes which disappeared within twenty-four hours. However, if the rabbit had been inoculated intravenously with staphylococci, streptococci or with pneumococci, and then injected with histamine, the leucocyte mobilization to the injected area continued for more than twenty-four hours. In control experiments in which phosphate solution was given instead of histamine there was no such mobilization. Findlay suggests that the localization of organisms in injured tissues may be due to the liberation in them of histamine or a histamine-like substance, which causes dilatation of the capillaries and increased permeability of the capillary walls.* The very interesting and fundamental question of the possible rôle of histamine or of a histamine-like substance in the production of the phenomena observed in inflammation is, therefore, still controversial. A thorough investigation of this subject is needed.

It would be anticipated that with the marked concentration of the blood there would be an increase in its viscosity. Waud (1928) has reported that the opposite holds true. Coincident with the fall in blood

*The fact that adenine compounds which cause a migration of leucocytes may be released under similar conditions (Bennet and Drury, 1931) was not appreciated when the work mentioned above was carried out.

pressure produced by histamine in the dog a decrease in the viscosity is stated to take place. This effect was not changed by rendering the blood non-coagulable with "heparin" or "novirudin." Waud had previously reported a reduction in viscosity of the blood in anaphylaxis.

In traumatic shock one of the characteristic signs is an acidosis. In a preliminary paper Wallace and Pellini (1920) stated that when histamine was given subcutaneously in repeated and eventually fatal doses there was an increase in the alkali reserve; but when given intravenously to the etherized animals, an acidosis was produced. Unfortunately these authors did not mention the animal employed. Underhill and Ringer, in 1921, while studying the alkali reserve in experimental shock produced by peptone and other agents, found that in dogs a mild acidosis was produced by histamine when it was administered in intravenous dosage of 1 to 2 mgm. per kilogram.

Hashimoto (1925) induced shock in dogs by the intravenous administration of 1 to 3 mgm. of histamine dichloride. For periods of three to five hours similar injections were made at intervals of 30 to 60 minutes. The animals were thus kept in shock for some time. In only one out of nine experiments was the shock severe enough to cause death. In this animal 70 mgm. of the salt had been injected. This particular animal showed a marked decrease in the carbon dioxide combining power of the plasma. In one of the other animals there was a rise in the alkali reserve, and in the balance of the animals no particular change was observed. The question arises, was the shock produced in the various animals of similar intensity? In the one dog which died in shock there was a marked decrease in the alkali reserve and it is possible this might have occurred in the others under similar conditions.

Hiller (1926) gave single doses of 1 to 3 mgm. subcutaneously to unanesthetized dogs. In all six of her experiments a mild acidosis resulted. At the same time the urine became more alkaline as a result of bicarbonate excretion.

The production of an acidosis by histamine injections has been confirmed by Boyd, Tweedy and Austin (1928), and by La Barre (1926). On the contrary Katzenelbogen (1929) found that small intramuscular injections of histamine raised the alkali reserve in rabbits and indeed suggested the use of histamine in treating human acidosis. There is conclusive evidence that in dogs, if not in rabbits, histamine causes an acidosis.

In anaphylactic shock in dogs the coagulability of the blood is diminished. This effect is not so marked in rabbits and guinea-pigs but is present in these species. In 1910 Dale and Laidlaw stated that hista-

mine had no effect on the coagulability of the blood. In 1911 they found that histamine given intravenously to a dog slightly retarded the clotting of the blood but not nearly to the same extent as is observed in peptone shock. Hashimoto (1925) found a definite reduction in the clotting time of dog blood after the injection of histamine. In investigations using rabbits M. I. Smith (1920) observed that blood from normal animals clotted in from five to seven minutes; the blood of rabbits in anaphylactic shock clotted in from fifteen to twenty minutes; the blood of animals in histamine shock clotted in either normal time or slightly less. Zunz and La Barre (1926) concluded that the clotting time of dog blood in histamine shock is about normal. These results while slightly conflicting, indicate that histamine shock does not affect the coagulability of the blood as does anaphylaxis. In anaphylactic shock there is no doubt that the blood clots much more slowly. In histamine shock there is practically no change from the normal.

A number of studies have been made on the effects of histamine on certain constituents of the blood.

1. *Chlorides.* Interest in this constituent is increased by the fact that the blood chloride is markedly decreased in acute intestinal obstruction and in certain infectious diseases. In four out of thirteen experiments on dogs Hashimoto (1925) observed a decrease in blood chloride after the administration of small doses of histamine intravenously. Drake and Tisdall (1926), also working on dogs, gave larger doses (up to 7.5 mgm.) subcutaneously. In a histamine intoxication lasting three to five hours there was no consistent change in plasma chlorides. But in prolonged intoxication there was a significant decrease. The conclusion has frequently been drawn that the lowered chloride in intestinal obstruction is due to loss of chloride from the stomach with frequent vomiting. Drake and Tisdall collected the vomitus from the dogs which had received histamine and found that the chloride collected from the stomach did not account for the loss of chloride from the blood. Their results clearly show an increased blood concentration and decrease in chloride. Saradjichvili and Rafflin (1930) gave one milligram of histamine intravenously or subcutaneously to human subjects and found that the blood chloride was lowered.

2. *Non-protein nitrogen.* In all his experiments on dogs Hashimoto (1925) consistently observed an increase in non-protein and urea nitrogen, the amount being from 50 to 94 per cent above the level maintained before the administration of histamine. The explanation that this increase was due to an increase in blood concentration occurred to Hashimoto but his determinations were made on plasma, and not on the whole

blood. He observed that during histamine shock the flow of urine was decreased. After recovery from shock the animal excreted abnormal amounts of urea and other nitrogenous substances. This indicated to Hashimoto that histamine had increased protein break-down in the animal. Subsequent histological examination of the kidneys showed degeneration of the tubular epithelium but not of the glomeruli. Hashimoto concluded that the observed increase in non-protein nitrogen in the blood was due, not to blood concentration but to increased protein break-down, to kidney damage, and diminished nitrogen excretion during shock. Hiller (1926) has stated that "histamine injection affords an easily controlled means of producing an acute transient condition of tissue destruction in the body." The increase in non-protein nitrogen in the blood has been confirmed by Drake and Tisdall.

3. *Protein.* Derer and Steffanutti (1930) found that in cats intravenous histamine increased the concentration of the blood, but in this concentrated blood there was actually less serum protein than in normal blood. This they attempted to explain on a basis of selective permeability of the vessel walls.

4. *Cholesterol.* After injection of histamine in dogs it has been found by Cornell (1928), and by Tangl and Recht (1928) that the blood cholesterol is decreased. The same condition exists after a meal is eaten, and Cornell has suggested that the decrease in blood cholesterol is associated with gastric activity, but not necessarily with gastric secretion. A comprehensive study of cholesterol secreted in bile during the lowering of blood cholesterol by histamine has not been made.

5. *Sugar.* Chambers and Thompson (1925) induced histamine shock in dogs anesthetized with morphine and ether by intravenous injection of 3 mgm. histamine per kilogram of body weight. Control determinations were carried out using the same anesthetic on dogs, which received no histamine. While this anesthesia alone produced an increased blood sugar the change was insignificant compared to that observed after histamine. During the first 30 minutes of histamine shock the blood sugar rose to 2 or 3 times the control level. This rise was accompanied by a decrease of 80 per cent in liver glycogen and a decrease of 60 per cent in the glycogen content of heart and skeletal muscle. La Barre (1926) observed similar results with guinea-pigs. Intravenous doses of histamine caused an increase in blood sugar accompanied by decreased liver glycogen. Atropinization of the animals appeared to prevent both the hypoglycemia and the decrease of liver glycogen, so La Barre suggested that the hyperglycemia was due to

stimulation of the vagus fibres distributed in the liver. Katzenelbogen and Abramson (1927) stated that in severe or fatal histamine shock there is a fatal hypoglycemia, while in mild shock there is a hyperglycemia. Menten and Krugh (1928) measured blood sugar changes in rabbits after intravenous administration of histamine. In some cases there was no change while in others there was a hyperglycemia. One may conclude from these various results that histamine does cause an increase in blood sugar. The question arises whether or not this change is due to something more significant than concentration of the blood. It may be that this explanation is adequate. On the other hand the observed changes in liver and muscle glycogen suggest other more specific but perhaps related effects.

6. *Lactic acid.* Chambers and Thompson (1925) in their studies on dogs secured an average increase of blood lactic acid of about 133 per cent during the first 30 minutes of histamine shock, followed by a fall to about 66 per cent above the normal by the end of about 60 minutes.

7. *Calcium.* La Barre (1926) found no change in plasma calcium in guinea pigs during histamine shock. Kuschinsky (1929), however, observed an increased amount of calcium in plasma and serum of dogs receiving large intravenous doses of histamine.

8. *Phosphate.* Chambers and Thompson (1925) noted a 50 per cent increase in plasma inorganic phosphate in dogs in histamine shock. In guinea-pigs La Barre (1926) found the opposite, i.e., a decrease in plasma inorganic phosphate.

9. *Lipase.* Steffanutti (1930) measured the lipase content of blood during histamine shock and found it to be lessened.

Histamine has so frequently been mentioned as a factor in anaphylaxis, in acute intestinal obstruction and in traumatic shock, that it is interesting to compare the blood changes in these states with those found in histamine shock.

Unfortunately complete information is not available regarding blood changes in traumatic and anaphylactic shocks. More complete data are available in intestinal obstruction. In details which are known the blood changes in histamine shock are identical with those in traumatic shock. The blood becomes more concentrated, due probably to loss of plasma. There is an acidosis and a marked increase in non-protein nitrogen. Histamine fails to give the appreciable change in clotting time of the blood which is found in anaphylactic shock. The blood changes in histamine shock are strikingly similar in many ways to those found in acute intestinal obstruction. Here again concentration of

the blood with increase in calcium, sugar and non-protein nitrogen is found. But in intestinal obstruction there is an alkalosis, in contrast with the acidosis resulting from histamine shock. So far as is known, histamine shock produces a condition of the blood which is similar to that found in traumatic shock, but which is not similar in all details to that in anaphylaxis and in acute intestinal obstruction.

To summarize this section—the most striking change in the blood in histamine shock is the increased concentration, and this is reasonably explained by the increased permeability of the vessel walls with loss of fluid into the tissues. There is an increase in the calcium, in the phosphate, in the non-protein nitrogen and in the sugar and it has been suggested that these result entirely from the concentration of the blood. While this may be an important factor there are other contributory causes, as is indicated by 1, the calcium increases are observed in the serum; 2, the increase in non-protein nitrogen is associated with increased protein catabolism, kidney damage and decreased urine excretion; 3, the increase in blood sugar is accompanied by loss of carbohydrate from the reserves. The diminution in blood chloride is in part attributable to secretion of gastric juice and that of cholesterol to bile formation.

HISTAMINE SHOCK. The early studies of Dale and Laidlaw (1910) and of Mellanby (1915) on the effects of histamine have already been referred to in some detail. We have considered it inadvisable to attempt to divide sharply considerations of the effects produced by large and small doses of histamine, and descriptions of the effect of large doses on various functions in several species are found under other headings. Dale and Laidlaw (1919) in their classical paper on histamine shock, reported the results of experiments designed to illustrate the effects of large doses of the amine on the blood pressure and blood volume of etherized cats. The rapid initial fall of blood pressure of 50 or 60 mm. is accompanied by a rise in pulmonary arterial pressure. (See effect of histamine on pulmonary arteries in cats.) This phase lasts only a few seconds. The right ventricle, which has been distended, returns to its normal state. The second phase, lasting 10 to 30 seconds, is a rise of pressure or a delay in the rate of fall. With the vagi intact there is a definite slowing of the heart at this stage. Dale and Laidlaw attribute this rise of pressure to arterial constriction. The third phase is the gradual fall of blood pressure to very low levels. The heart beat becomes very faint, the body temperature falls, and no more anesthetic is required. When histamine was infused slowly, the first effect was a sharp fall in pressure, followed by a further slow descent. The first

and second stages of the effect produced by rapid injection of histamine were not apparent after the slow infusion. In the further study of the cause of the failure of the circulation, decreased cardiac output attributable to diminished venous inflow was demonstrated. As has been discussed elsewhere, the heart continued to beat forcibly, and the blood pressure could be raised by the intravenous administration of fluid. Since the great veins were empty the search for the lost blood was initiated. In the cat there is little or no obstruction to the hepatic circulation in histamine shock, and Dale and Laidlaw eventually found that the cause of the diminished volume of circulated blood in this species was leakage of the plasma in the peripheral tissue spaces. This produced a reduction of blood volume, and an increased concentration of the cellular elements of the blood. The main factor in the failure of the circulation, however, is the reduction of the active blood volume produced by the capillary dilatation and the resulting stagnation of the blood in the periphery. The changes in the blood chemistry in histamine shock in the cat have been reviewed in another section.

In the cat the liver plays a very little part in the production of histamine shock. In the dog the liver plays a more prominent rôle, though in this species also peripheral vasodilatation is an important factor. It is unlikely that liver blockade plays an important part in the lowering of blood pressure produced by histamine in the monkey.

SECONDARY WOUND SHOCK AND TRAUMATIC SHOCK. The great significance of secondary wound shock in human individuals during the Great War, and the similarity between the signs and symptoms of this condition and those of histamine shock in animals has led to the supposition that the two conditions are produced by similar mechanisms. The reviews of Dale (1919), and Cannon (1919), may be consulted for further details. The clinical researches of Quénu (1918), and of Delbet (1918), emphasized the similarity of the symptoms of wound shock to histamine poisoning. Quénu believed that wound shock could be prevented by the immediate removal of lacerated tissues. Delbet found that extracts of autolysed muscle produced shock-like conditions in animals. Turck (1929) has reported that bruised tissues removed from an animal and transplanted to another may produce death in the recipient, while the donor survives. Bayliss and Cannon (1919) have demonstrated that a shock-like condition can be produced experimentally by crushing limb muscles of anesthetized animals. This effect occurred when the nervous connections of the limb were severed. Hoet (1929) was unable to confirm this result. The fall in blood pressure in Bayliss

and Cannon's experiments was not observed when the blood vessels to the limb were ligated, and the nervous connections were intact. When the ligatures were untied from the vessels, a shock-like condition was found to develop immediately. Investigations of the blood lost into the bruised tissues appeared to show that the amount was not sufficient to account for the fall of blood pressure. These experimental results have been widely quoted in favour of the theory that a histamine-like substance is formed in bruised tissues, and that its absorption is responsible for traumatic shock in animals, and secondary wound shock in human beings. Although the work of Lewis and his collaborators provides evidence which supports the view that appreciable quantities of a histamine-like vasodilator substance would be liberated by such extensive damage to skin and deeper tissues as is produced in traumatic shock, yet it should be emphasized that there is no direct evidence that the amount of vasodilator material in the traumatized area is increased. Brief reference may be made here to the work of Edwards (1920) who, in a study of the pulse curve after histamine and after vascular occlusion, found many features in common, but he did not believe that more than a small part of the occlusion effects were attributable to the formation of histamine or histamine-like substances. He obtained no evidence that a vasodilator substance was carried into the general circulation when the blood flow through the occluded area was re-established. Very large doses of histamine were required to initiate circulatory changes comparable to those produced by occlusion. The more recent work of Phemister and Handy (1927), which is referred to elsewhere in this review, is also very interesting in this connection.

While no attempt will be made to review the literature on secondary wound shock, or on traumatic shock in animals, the experiments of Smith (1928), and of Blalock (1930) may be discussed briefly. Smith ligated both artery and vein of the limb of an etherized dog. He then traumatized the muscles of the limb, and an hour and fifteen minutes later he unclipped the artery, and removed 25 cc. of blood from the distal end of the vein. The blood pressure fell 20 mm. of mercury. This blood was then injected into the femoral vein of the opposite side, and blood pressure returned to normal. There was, therefore, absolutely no evidence of any toxic substance in the blood from the traumatized area. Blalock, using dogs under barbital anesthesia, has shown when the vein in the traumatized limb of an animal is allowed to discharge its blood into the circulation, no fall of blood pressure is produced after blood flow to the limb is prevented by means of a tourniquet. Blalock sug-

gests that the failure of previous workers to recognize that hæmorrhage alone is responsible for the signs of traumatic shock, is attributable to the difficulties in obtaining an accurate estimation of the amount of blood lost in the traumatized limb.

These experiments provide very strong evidence that the fall in blood pressure in traumatic shock in animals is attributable to loss of blood into the damaged tissue, and not to the absorption of toxic substances.

BURNS. Extensive burns in human subjects and in animals may be followed by a state of shock which has certain points in common with the condition produced in animals by large doses of histamine. The concentration of the blood and the changes in blood chemistry are, in many respects, similar. An increased secretion of epinephrine and a decreased amount in the adrenal glands of cats have been reported to be produced by superficial burns (Hartman, Rose and Smith 1926). Although Robertson and Boyd (1923) extracted from burnt skin substances which produce a shock-like condition in normal animals, there is as yet no evidence that these toxic substances are absorbed or that they possess more than a superficial resemblance to histamine. Reference is made elsewhere to Harris' finding that the vasodilator material disappears from the skin of a scalded area. Bennet and Drury (1931) believe that burning liberates a histamine-like substance from cardiac muscle. Underhill's recent evidence that water loss through the burned surface and in edema is sufficient largely to account for the concentration of the blood, is also very interesting. We are not yet in a position to decide on the relative merits of the theories advanced to explain the toxemia sometimes produced by burns.

ACTION ON THE FROG. The effect of histamine on the frog as originally noted by Dale and Laidlaw (1910) is very slight. There is a slight rise of blood pressure attributable to increased cardiac activity after intravenous administration of the amine (Dale and Laidlaw, 1911). Several facts make many of the reported results difficult to evaluate. In some cases the histamine solution used was not neutralized, while in others the species of frog used or the season of the year in which the experiments were made is not specified. The effect of neutral histamine solution on the heart of the various species of frogs has not been studied sufficiently to justify any more definite conclusions than that small or moderate amounts have either no action or a slight stimulating effect and that massive doses inhibit the activity of the heart. These problems have been investigated by Einis (1913), Rothlin (1920), Lio (1927) and others. The well-controlled studies of Grant and Jones (1929) on *R. esculenta* and *R. temporaria* in which a wide range of histamine dos-

age was used, failed to reveal any vasodilator effect of the amine. In *R. temporaria*, large doses produced an increase in the strength of the heart beat and in blood pressure. The depressor effects reported by Doi (1920) were not observed.

The capillary circulation in the frog has been studied by numerous investigators. Doi (1920) and Killian (1925) reported that the capillaries of the frog's web and tongue were dilated by histamine. Fraser (1929) obtained dilatation of the capillaries in the tongue of *R. pipiens* in one instance, but not in six other animals studied. Grant and Wood (1928) and Grant and Jones (1929) failed to obtain any capillary dilatation in the peritoneum, nictitating membrane, or tongue of frogs. *R. esculenta* and *R. temporaria* were used and unanesthetized, decerebrate, and anesthetized animals were studied. Krogh and Rehberg, and more recently Drinker (1929) using *R. temporaria* failed to find any loss of capillary tone when histamine is added to perfusion fluids. Krogh (1929) states that he is "confident that histamine even in high concentrations has no action on frog's capillaries." Clark (1924) concluded that histamine produced a constriction of the capillaries when the pH of the perfusion fluid was kept constant.

The lack of action of histamine on the frog's capillaries, which respond readily and in a manner similar to mammalian capillaries to mechanical and other stimuli, is made clearer by the findings of Grant and Jones (1929). An extract of frog skin was shown to contain a substance which produced capillary dilatation in the frog's tongue. Histamine and extracts of mammalian skin were without effect. It appears, therefore, that the frog is provided with a vasodilator substance, which is not histamine. This vasodilator material has been shown to resemble histamine chemically and physiologically.

ACTION ON THE BLOOD PRESSURE OF THE RABBIT. The action of histamine on the blood pressure of the rabbit is of some special interest because it was supposed for some time that the amine did not produce peripheral vasodilatation in this species.

Ackermann and Kutscher (1910) and Dale and Laidlaw (1910) noted that rabbits under the usual anesthetics did not respond with a fall in blood pressure to the intravenous administration of small amounts of histamine. After large doses Dale and Laidlaw recorded a fall of blood pressure which was due to effect on the pulmonary circulation. The studies of Lewis and Marvin (1927) demonstrate that the local effects of histamine on the rabbit's conjunctiva are similar to those observed in other species in which the amine produces peripheral vasodilatation.

Feldberg (1927) established the fact that the anesthetic used may determine the response of the blood pressure to histamine in this animal. In rabbits under chloralose histamine usually produces capillary dilatation and slight arterial constriction. The blood pressure falls. With large doses arterial constriction may be the predominant effect. Dixon and Hoyle (1930) have found considerable individual variation in the response of rabbits, which is independent of the depth and type of anesthesia. In the denervated ear of the unanesthetized rabbit histamine injected intravenously produces capillary dilatation and constriction of the central artery (Feldberg, 1927). The veins are also dilated. Flatow (1929) has confirmed this capillary dilator effect but he did not always observe constriction of the central artery.

Histamine constricts the coronary and pulmonary arteries in the rabbit, but exerts little effect on the liver vessels. The right auricular pressure is raised during the period of action of histamine (Dixon and Hoyle, 1930).

Although the type of anesthetic greatly modifies the action of histamine in this species, rabbits even under the most favourable conditions are not nearly as sensitive as the carnivorous animals which have been studied.

Effect of anesthesia. Dale and Laidlaw found that unanesthetized cats could tolerate very large doses of histamine. Cats under ether were much more susceptible. This increase of susceptibility persisted for 24 hours or longer after recovery from the anesthetic. Rabbits and guinea-pigs were less sensitive to histamine under anesthesia than under normal conditions. Dale (1920) in extending this work found that nitrous oxide did not produce an increased susceptibility to histamine in cats. This difference in the effects of ether and nitrous oxide is particularly interesting in light of the observations made during the War, which showed that ether anesthesia in human patients was much more likely to be followed by secondary wound shock than was nitrous oxide. Experimental observations demonstrating that the action of histamine may be greatly modified by changing the depth or type of anesthesia have been referred to under various headings. The observations of McDowall (1923) on pulmonary vessels, Feldberg (1927) on the fall in blood pressure in rabbits, Forbes, Wolff and Cobb (1929) on cerebral circulation and cerebrospinal fluid pressure, and of Dixon and Hoyle (1930) on pulmonary circulation and others have been mentioned. Very little further discussion of this aspect of the subject is necessary here.

The histamine contraction of smooth muscle is inhibited by narcotics. Katz (1929) has compared the effects of chloral hydrate, urethane and ether on the contraction produced by histamine of strips from various blood vessels. Small concentrations of chloral hydrate inhibited the action of histamine. Urethane caused a very powerful contraction of the vessel strips. This made it difficult to investigate the effect of histamine, but in certain cases it was possible to show that the action of histamine was decreased. Ether also inhibited the action of the amine on strips of smooth muscle.

ACTION ON HUMAN SUBJECTS. References to effects of histamine on human subjects have been made under various headings in this review. To avoid repetition we have included in this section only data which have not been discussed in detail elsewhere.

Kehrer (1912) reported that he had observed convulsions, temporary cessation of respiration, and unconsciousness in patients after large doses of histamine. Eppinger and Gutman (1913; also Eppinger, 1913) noticed a fall in blood pressure and blushing produced by the subcutaneous injection of the amine.

In 1913 Jaeger reported the use of histamine in obstetrical cases. He found that the subcutaneous or intramuscular injection of the amine increased labor pains and hastened the birth. Very large doses were required, however, and flushing of the skin of the head and neck, palpitation of the heart, and vomiting were produced. He reported also that the oral administration of histamine accelerated the post-partum involution of the uterus. In another paper Jaeger (1913) reported the results of the subcutaneous administration of mixtures consisting of various proportions of histamine and *p*-oxyphenylethylamine. By reducing the proportion of histamine the disagreeable effects of the larger amounts were eliminated. This mixture was reported to be most effective when given after parturition. The reports that oral administration of histamine affects the uterus have not been confirmed and are not convincing.

Schenk (1921) has described in some detail the signs and symptoms produced in man by histamine. Doses of 2 to 8 mgm. given subcutaneously were used. The flushing of the skin of the head and neck was intense. Nausea and spasms of the bronchial musculature were produced. The fall in blood pressure was in some cases more profound than safe. For example, in a 50 year old patient the initial pressure was 197/100 mm. Hg. After 4 mgm. of histamine the pressure fell to 80/45 mm. Hg. After 80 minutes the levels were 135/70 mm. Hg. Double

this dose (i.e., 8 mgm.) produced arrythmia and ventricular extrasystoles in a patient 51 years old. Under the x-ray a very complete contraction of the stomach was observed after doses of from 6 to 8 mgm. The action of histamine could be prevented by the simultaneous administration of epinephrine. Fifteen milligrams of histamine and 1.5 mgm. of epinephrine could be injected without the production of deleterious effects.

Harmer and Harris (1926) obtained very definite effects of histamine in man with very much smaller doses than are usually used in animals. One-twentieth milligram of histamine hydrochloride was administered intravenously. This amount produced an increased cutaneous blood flow. The reddening of the skin and the rise in skin temperature were quite definite. Reddening of skin previously made cyanotic by congestion suggested that the arteries supplying the skin were also dilated, but no evidence of increased blood supply to the muscles was obtained with these small doses. A small increase in limb volume was noted. The systolic and diastolic blood pressure fell slightly. The rate of transudation of fluid, measured by the plethysmographic method of Drury was definitely increased above the normal by injection of histamine. As Harmer and Harris kept the pressure in the small vessels constant by means of a blood pressure cuff, the rise in the rate of transudation was attributable to increased permeability of the vessels. These small doses produced a slight acceleration of the pulse rate but no perceptible change in respiration.

Weiss, Robb and Blumgart (1929) have utilized the time interval between the intravenous injection of a small amount of histamine (0.001 mgm. of histamine phosphate per kilogram in a dilution of 1:5,000) into the arm vein and the appearance of a flush on the face as a measure of the efficiency of the circulation. The interval is presumably a measure of the time required for the blood carrying histamine to travel from the arm to the right heart, through the pulmonary circulation and to the face. This "histamine reaction time" has been determined in 65 normal individuals and in numerous pathological conditions. The results of repeated tests on the same individual agree well. A definite peculiar sensation is felt in the tongue at approximately the same time as the flush appears and there is cardiac acceleration. Electrocardiographic records demonstrated a temporary inversion of the T wave in certain subjects. Patients with circulatory failure showed a prolonged reaction time. In individuals with pernicious anemia or hyperthyroidism the interval was greatly decreased, i.e., the blood flow was more rapid.

Patients with pulmonary disturbances or with symptoms of myocardial failure should not be given the usual amount of histamine, as bronchial spasm or other disturbing effects may be produced.

Bartels and Powelson (1929) used the same method as Weiss, Robb and Blumgart, i.e., 0.1 mgm. of histamine was injected into the vein of the arm or leg, and the time for the flush to appear on the face was determined. Forty cases of vascular disease were studied. Certain individuals were particularly susceptible to the amine. The "histamine reaction time" in normal subjects was between 17 and 25 seconds. In sixteen cases of thrombo-angiitis obliterans the interval was from 22 to 68 seconds. In arteriosclerotics the time was between 31 and 52 seconds. The circulation rate as measured by this method was practically normal in Raynaud's disease. In these patients the cyanotic areas showed after histamine patchy red warm spots and white cooler areas. Bartels and Powelson state that histamine did not produce these changes after removal of the appropriate sympathetic ganglia.

Euler and Liljestrand (1928), and Kisch (1929) observed an increase in minute volume of the heart after the subcutaneous injection of small doses of histamine to human subjects. Weiss, Ellis and Robb (1929) have reported an interesting series of observations on the effects of histamine slowly administered intravenously to human subjects. Circulatory changes were produced when 0.0003 mgm. of the amine per minute was injected. Adult individuals were given histamine at the rate of 0.02 to 0.04 mgm. per minute for periods up to two hours. The heart rate, cardiac output, velocity of blood flow, pulmonary ventilation and metabolic rate were increased. There was no fall of blood pressure with an injection rate of 0.02 mgm. per minute. The blood volume was not changed. The stroke volume of the heart and the mean velocity of blood flow were decreased. The amount of epinephrine necessary to counteract a given dose of histamine varied with the different effects of histamine. The reddening of the skin was found to be due to dilatation of the venules as well as the capillaries and arterioles. Blumgart (1931) has recently reviewed the work on the use of histamine in studies of the circulation time. The advantages and disadvantages of the "histamine method" are discussed. Fényes (1930) has recently reported the results of the subcutaneous ($\frac{1}{2}$–1 mgm.) and oral (2–12 mgm.) administration of histamine to human subjects. The effects of the subcutaneous injections were similar to those obtained by others. There was an increase in volume, free HCl and total acidity of the gastric juice. An increased red cell count and hemoglobin content of the blood was observed as long as

three hours after the injection. The local skin reaction and effects on the blood pressure and heart rate were typical. The most interesting feature of this paper is the report that oral administration of these relatively small amounts of the amine produced an increased secretion of gastric juice and bile and an increased hemoglobin content and red cell count of the blood. The figures given are quite convincing, but the effects of these small amounts given by the oral route are much greater than those secured by other workers.

Information on the objective effects of histamine on human subjects has accumulated very rapidly during the last few years. A large proportion of these data relates to effects on gastric secretion, and it has been considered under that heading. A few comments on the sensations experienced after small and moderate doses administered intravenously may be included here. The rate of injection affects the intensity of the sensations to a much greater extent than the total amount given. A rapid injection of 0.015 mgm. of the base may produce a very definite metallic taste, a feeling of increased temperature, particularly in the face and neck, a feeling of tightness in the head, and a headache which may last for several hours. With larger doses (0.15 to 0.2 mgm. administered within ten seconds) a very intense metallic taste was the first symptom noted in our personal experience. This persisted for only a few minutes. The flush was felt some 5 to 10 seconds later and was noted by observers a few seconds after that. The sensation of tightness in the head was very uncomfortable and was accompanied by ocular disturbances. Numerous rods of white or silver coloured light, which rapidly changed their positions but which usually appeared to converge on a point approximately in the centre of the field, were visualized. The headache, which may be intense, may persist for several days. The feeling of tightness in the head and the headache may be attributed to increased intracerebral pressure. The ocular disturbances may be associated with increased intraocular pressure or possibly with dilatation of retinal blood vessels. The peculiar metallic taste varies in different individuals. Further investigation of these phenomena should provide more adequate explanations of the mechanism of their production.

VASCULAR REACTIONS OF THE HUMAN SKIN. One of the most interesting aspects of the action of histamine is the effect produced when minute amounts are introduced intradermally. This effect was first noted by Eppinger (1913). It has been studied in various animals by Sollmann and Pilcher (1917) and other workers, but the most com-

prehensive investigations are those of Lewis and his collaborators (1927). Since it would be impossible to do justice to this subject in the space available and since Lewis' work and the recent important discussions of it by Krogh (1929) and Dale (1929) are readily available in English, only a very brief summary of the more significant results will be given here. The effects produced by the intradermal injection of a very small amount of 1:1000 solution of histamine phosphate are essentially similar to those produced by a variety of mechanical or other chemical stimuli. The reaction consists of three separate phenomena which are collectively termed "the triple response." These are 1, vasodilatation of the small vessels at the site of injection; 2, a diffuse flare which may have a diameter of three or more centimeters; and 3, an area of local edema which appears soon after the beginning of the flare and over the same area which exhibited the vasodilatation. The maximal diameter of the wheal is usually reached within five minutes after the injection. The wheal usually disappears within an hour. When the nerves to the skin have degenerated, the wheal still may be elicited but not the flare. Lewis has demonstrated that the exudation of fluid forming the wheal is produced by increased permeability of the vessel wall and not by increased filtration pressure. When the increased permeability exists the rate of exudation is proportional to the blood flow.

There is a remarkable similarity in time relationships of the appearance and disappearance of the three parts of the triple response elicited by pricking histamine into the skin and that produced by a mechanical stimulus. It has been shown that raising the temperature of the skin or arresting the circulation favours the development of more violent and complex vascular reactions. Lewis suggests that this is due to accumulation of normal metabolites through increased rate of formation or decreased rate of removal. When the stimuli pass to the skin in the sensory nerve fibres, as in the case of antidromic impulses, the channel of these impulses may be considered physiological, and since the diffusible substance has been shown to accumulate under these conditions the evidence that this is a normal metabolite is reinforced. The properties of this diffusible substance resemble those of histamine to such an extent that Lewis has stated that the conclusion that histamine in loose combination or free is identical with the H-substance "would harmonize with the chief evidence at all points." Having in mind the prolonged effects of the H-substance, which are not readily attributable to such a diffusible substance as histamine, and the possibility that unidentified disintegration products of tissue may also play a rôle in the

vascular response to skin injury, Lewis prefers to retain the term H-substance. Lewis has shown that the simultaneous production of wheals at various points on the skin of a subject exhibiting factitious urticaria may produce a blushing of the face similar in every respect to that obtained after the subcutaneous injection of histamine. As mentioned elsewhere, Kalk has demonstrated increased gastric secretion after mechanical irritation of the skin in these sensitive subjects. Although histamine has not been isolated from extracts of skin its presence therein is made probable by the work of Harris, who demonstrated the presence of an active vasodilator material which possessed many of the physiological properties of histamine.

It seems feasible, therefore, to suppose, as Dale (1929) has suggested, that Lewis' H-substance owes its principal physiological activities to the presence of histamine but that the action of histamine liberated from the cells may be modified to a varying extent by the loose combination with it of other substances.

Krogh (1929) accepts without reservation the hypothesis that histamine or a substance with similar physiological properties is liberated from mammalian skin whenever the cells are damaged. Lewis' thesis that tissue cells in general may release a substance having a dilator action on the minute vessels is also accepted, but Krogh finds it impossible to accept the suggestion that only one chemical substance is responsible for the complete triple response. He has suggested the term H-colloid to indicate the possibility of the presence of a less diffusible vasodilator. If the H-substance is one chemical substance it is necessary to suppose that it acts on nerve cells to produce liberation of itself from other tissue cells. That another vasodilator material may play a rôle in the triple response has been suggested by Dale (1929) who with Dudley (1929) has recently isolated acetylcholine from fresh normal tissue.

The cutaneous reactions to intradermal administration of histamine in certain pathological conditions have been studied by Lewis and his collaborators and by various others workers (Villaret and Justin-Besançon, 1926; Caldwell and Mayo, 1931), but as we have only outlined the physiological results on which this work is based, a discussion of these findings will not be included here.

EFFECT OF HISTAMINE ON SMOOTH MUSCLE. A great deal of the information we now possess regarding the action of histamine on smooth muscle is to be found in the paper by Dale and Laidlaw (1910).

Bronchioles. The profound bronchial constriction produced in cer-

tain rodents by histamine is the most noticeable effect. Bronchial spasm is also observed in cats but the intensity is in most instances much less. Atropine does not relieve the bronchial spasm.

In normal guinea pigs a dose of 0.1 mgm. of histamine intravenously may produce intense spasm of the bronchial muscle and death. The heart may beat for some time after respiration ceases. The lungs do not collapse post mortem but protrude from the thoracic cavity when this is opened. Under the microscope the bronchioles are seen to be strongly contracted. The alveoli are distended with air. Somewhat similar effects are observed in rabbits, while in mice and rats and in dogs and cats, as has been discussed elsewhere, the circulatory effects are much more prominent than the pulmonary. Histamine constricts the bronchioles in spinal animals (Dale and Laidlaw, 1910; Koessler and Lewis, 1927). In cross circulation experiments Houssay and Cruciani (1929) separated the head of a dog from its body so that only the vagi provided connection between the two parts. The separated head received its circulation from another animal. Injection of histamine into the decapitated animal produced bronchial constriction but no effect was secured in this animal when histamine was added to the blood supplying the head. The denervated dog lung may be less sensitive to histamine (Weber, 1914) but the denervated lungs of other animals react to small amounts of the amine. The peripheral effects of histamine on the bronchioles are apparently much more important than the central. In isolated perfused lungs of the dog or cat histamine produces bronchial constriction.

Large doses of histamine in man produce respiratory distress attributable to bronchial constriction (Schenk, 1921) while after small amounts respiratory effects are not observed (Weiss, Ellis and Robb, 1929).

Uterus. The action of histamine on the uterus is of particular interest since this was the first physiological effect of the amine observed. An isolated horn of the uterus of the virgin guinea-pig was found by Dale and Laidlaw to be a very satisfactory test object for histamine. A distinct effect on the uterus was produced when 1 part of the amine was present in 250,000,000 of Ringer's solution. The sensitivity of the uterus varied with the age of the animal and the condition of the uterus. Large doses of histamine produced contraction of the uterus *in situ* (cat and guinea pig). The uterus in the latter stages of pregnancy was much less sensitive to the amine. The uterus contracts as a whole when stimulated by histamine and an expulsive action is not demonstrable. This action of histamine is not altered by atropine and apparently is on the muscle fibres directly.

As mentioned previously the suggestion was made that the oxytocic activity of pituitary preparations could be standardized against histamine. It was found, however, by Trendelenburg and Borgmann (1920), and by Burn and Dale (1922), that the change in susceptibility of the test object during an experiment may not be the same for histamine and the oxytocic principle of the pituitary gland. The concentration of the salts in the Ringer's solution may affect the susceptibility of the isolated uterus to histamine (Dale, 1913). Increase in the concentration of the salts decreases the sensitivity of the uterus. Cane sugar or sodium sulphate produce similar effects. This effect appears to be due to change in the osmotic pressure of the fluid surrounding the uterus. Very high concentrations of histamine inhibit the uterus (Abel and Macht, 1919). The isolated uterus of other species also responds to histamine by contraction (dogs, Quagliariello, 1914), (rabbit, Feldberg and Schilf, 1930). In the mouse and rat, however, the action of histamine on the isolated uterus is not consistently to produce a contraction. The effect on the mouse uterus varies with the concentration of histamine. Abel and Macht (1919) have shown that there is a fairly wide range of concentration in which histamine produces little or no effect on the mouse uterus. Lower concentration stimulates while higher ones inhibit the contraction of the uterus. The conflicting results of Adler (1918), and of Cow (1919), are attributable to the different concentrations of histamine used.

With the rat's uterus inhibition of the contractions is usually produced by histamine and this result may provide useful information in the physiological identification of the amine. This action was first observed by Guggenheim (1912) and the result has been repeatedly confirmed (Fühner, 1912; Longcope, 1922, and Voegtlin and Dyer, 1924). Abel and Macht (1919) also observed an inhibition but with very small doses a slight increase in tone was obtained. Quite recently Kellaway (1930) has shown that the normal rat uterus or one passively sensitized to a foreign protein responds to histamine by relaxation. Since the sensitized uterus responds to the specific antigen by contraction Kellaway contends that histamine can play no part in this anaphylactic phenomenon. This paper has been discussed under "Anaphylaxis."

Contraction of the uterus *in situ* produced by histamine has been observed in several species. Dale and Laidlaw (1910) recorded the contraction of the uterus of the pithed cat after 1 mgm. of histamine administered intravenously. In the pregnant cat the uterus is less sensitive but contractions are produced and separation of the placenta may

occur. In pithed dogs Jackson and Mills (1919) compared the effects of histamine and pituitary extract upon the uterus. Some of the earlier pituitary preparations owed part of their uterus-stimulating effect to histamine. Hofbauer and Geiling (1926) have studied the effects of histamine administered intravenously or intracardially to pregnant guinea-pigs and cats. The immediate effect was a tetanic contraction of the uterus with engorgement of its vessels and those of the broad ligament. In guinea pigs but not in cats, the placenta showed signs of separation. Thrombi in the veins of the decidua and in those of the liver and other pathological changes were observed in both species. Some of these changes were similar to those found in human eclamptic patients. Hofbauer (1926) has developed this thesis further and pointed out the similarity in the blood chemistry in eclampsia in humans and acute histamine poisoning in guinea pigs. In the case of eclampsia, as in various other conditions we have considered, there is as yet complete absence of satisfactory direct evidence that histamine plays any significant rôle.

A number of observations on the effect of histamine on the human uterus are discussed under "Effect on human subjects." Bourne and Burn (1927) have secured very interesting results in pregnant women during labour by determining the increase in pressure within the uterus after the subcutaneous administration of histamine. Doses of $\frac{1}{3}$ to 2 mgm. of the base were given. A prompt increase in pressure was obtained after the larger doses and the effect observed for as long as 50 minutes. After the effect of histamine had worn off the activity of the uterus ceased completely for a time. This suggested that the uterine muscle was excessively fatigued by the histamine stimulation. Labour was not accelerated by the uterine contractions produced by histamine. It is established therefore, that histamine has no place in the induction or acceleration of labour. It may be useful, as Bourne and Burn have suggested, to produce promptly a spasm of the uterus post-partum. Histamine combined with ergotoxin should produce a prompt and prolonged effect since ergotoxin after a latent period of some 20 to 30 minutes (Bourne and Burn) may stimulate the uterus to contract for several hours.

Intestine. Dale and Laidlaw (1910) found that large doses of histamine intravenously produced vomiting and purging of the unanesthetized cat. Smaller doses in anesthetized or pithed cats produced augmentation of rhythm and apparently also of the tone of the intestine. The isolated jejunum of the cat was shown to be intensely stimulated

by a concentration of 1:500,000 of histamine in a saline bath in which the intestine was suspended. The effect was not abolished by atropine. Quagliariello (1914) using isolated rings of dog and rabbit intestine noted a strong contraction of the musculature after the addition of histamine. Vanysek (1914) has studied the action of histamine on the isolated intestine of cats. Histamine stimulated to contraction intestine inhibited by magnesium chloride or chloroform.

Guggenheim and Löffler (1916) have studied the effect of histamine on the isolated small intestine of rabbits and guinea-pigs. An augmentation of contractions and tone was observed in both species. The guinea-pig intestine was shown to be particularly sensitive to histamine and a concentration of 1 in 250,000,000 of histamine hydrochloride produced a definite effect. This reaction provides a delicate physiological test for the presence of the amine. Olivecrona (1920) reported the results of an interesting series of experiments on the effects of large amounts of histamine on the small intestine of cats and rabbits. In cats the rhythmic contractions and tonus were inhibited by the doses used (1:750,000 and higher), but the inhibiting effect was always preceded by a transient increase in tonus. On rabbit intestine an inhibition of tonus without preliminary stimulation was noted. When the intestine of either animal was treated with an amount of histamine which produced complete inhibition a reappearance of tonus and rhythmic contractions after intervals varying directly with the amount of histamine used, was noted. The explanation of this effect is not clear. Mellanby's results (1915) suggest that the histamine does not disappear.

In an isolated loop of cat intestine in which the normal blood supply was retained (Lim and Chen, 1926) contraction was observed after the local application or systemic intravenous administration of histamine. In the isolated cat intestine from which the ganglion-containing tissue had been removed, Gasser (1926) observed a contraction after histamine in a few instances.

Bishop and Kendall (1928) and Kendall and Bishop (1928) have shown that histamine applied to the outside of guinea-pig intestine increased the sensitivity of the gut to electrical stimulation. The longitudinal muscle was found to be more susceptible to the effect of the amine than the circular. When histamine was added to the fluid used to irrigate internally a segment of isolated intestine, large amounts produced shortening while smaller doses restored rhythm and response to electrical stimuli. Kendall and Shumate (1930), found the distal end of ileum of guinea-pig gut to be the most responsibe part to histamine

stimulation. The large intestine responded sluggishly. Crane and Henderson (1924) found the ileum of guinea-pigs more sensitive to increase of internal pressure than other parts of the gut. Other interesting results recorded by Kendall and his collaborators have been referred to under "Anaphylaxis" and "Inactivation of histamine."

Ivy and Vloedmann (1923) found no effect on the movements of the stomach and intestine of dogs after small intravenous doses of histamine. MacKay (1930) found that the intravenous administration of histamine to dogs and cats ($\frac{1}{4}$–$\frac{1}{2}$ mgm.) provoked a motor reaction in the intestine, which was usually most marked in the ileum and which became less as the duodenum was approached. Similar effects were produced by the local application of histamine to the outside of the intestine. Atropine diminished but did not abolish these effects of histamine. Large doses of the amine placed in the lumen of the gut did not produce any appreciable effect on motility. Small amounts (1 mgm.) administered subcutaneously were also without demonstrable effect. Other observers (Gruber and Robinson, 1929, and Nechoroschew, 1929) have reported an increase of the movements of the stomach and intestine. In unanesthetized dogs moderate amounts of histamine may produce visible increase in peristalsis. In human subjects vomiting and purging have been reported and Schenk (1921) has observed under the x-ray, as noted elsewhere, contraction of the stomach produced by histamine.

Action on the gall bladder. The action of histamine on the isolated gall bladder is consistent with its effects on smooth muscle in general. Brugsch and Horsters (1926) and Halpert and Lewis (1930) demonstrated that the amine produces contraction of the isolated gall bladder of the dog. Brugsch and Horsters (1926), Erbsen and Damm (1927), Erbsen (1928) and Kalk (1928) have reported that the isolated gall bladder of the guinea-pig is contracted by histamine. Brugsch and Horsters (1926) found that large doses of histamine (10 mgm.) caused a contraction of the gall bladder in dogs. Ivy and Oldberg (1928) found no change in gall bladder pressure in cats after an intravenous dose of histamine which caused a marked decrease in blood pressure. In the dog, however, every agent, including histamine, which lowered blood pressure produced a rise in gall bladder pressure. As the introduction of hydrochloric acid into the duodenum produces a contraction of the gall bladder of the dog, this mechanism may also be operating. It has been found, however, by Lueth, Orndoff and Ivy (1929) that the subcutaneous administration of histamine to dogs or to human subjects was followed only occasionally by evacuation of the gall bladder. Fail-

ure of the acid produced in the stomach to reach the duodenum in sufficient quantities, and contraction of the muscle controlling the escape of bile into the duodenum, are suggested as explanations of the inconstancy of action of histamine. Bradburn and Blalock (Blalock, 1930) found that the intravenous injection of large doses of histamine in dogs under barbital produced definite changes in the gall bladder. The organ decreased in size and contained less bile. The bluish tinge was replaced by a whitish colour. The walls were thickened and microscopic examination revealed considerable edema.

Smooth muscle of other organs. Dale and Laidlaw (1910) noticed that histamine produced contraction of the spleen in cats. They noted that the spleen volume returned to normal while the blood pressure was still very low. Voegtlin and Dyer (1924) found enlarged spleens in mice which were killed with large doses of histamine. Barcroft, Harris, Orahovats and Weiss (1925) reported that the decrease in weight of the spleen observed in cats after death produced by histamine was appreciably less than that usually found when the animals were killed by coal gas, poisoning, drowning or hemorrhage. Dale and Laidlaw found little or no direct effect on the muscle of the urinary bladder of cats. When the nerves to the bladder were intact moderate doses of histamine produced contractions of the viscus and evacuation of its contents. The retractor penis of the dog and goat was contracted by histamine.

Effect on the eye. In anesthetized animals Dale and Laidlaw (1910) observed no change in the size of the pupil after the intravenous administration of 0.5 mgm. of histamine. In the unanesthetized cat large doses produced a constriction of the pupil, but there was no evidence that this was a direct effect on the muscle of the sphincter. Hamburger (1926) reported that small amounts of histamine introduced into the human conjunctival sac produced a miosis. Based on this observation histamine has been used in the treatment of glaucoma, but there seems to be no good reason for its use in preference to other more powerful miotics. Matsuda (1929) has studied the effect of injections of histamine *a*, into the carotid artery; *b*, into the anterior chamber of the eye; *c*, subconjunctivally, and *d*, into the conjunctival sac. In the rabbit a constriction of the pupil was observed after the intra-arterial injection, while in the dog and cat there was dilatation. In cats no effect was produced by dropping histamine solution into the conjunctival sac or injecting it subconjunctivally. The pupil increased in size after injection of the amine into the anterior chamber. In the dog, constriction of the pupil followed injection of histamine into the anterior chamber,

while in the rabbit administration of the amine by any of these routes produced constriction.

Very recently Colle, Duke-Elder and Duke-Elder (1931) have studied the changes in intraocular pressure in dogs and cats produced by the intravenous administration of from 0.05 to 0.005 mgm. of histamine. In the intact animals the fall in systemic pressure was invariably accompanied by a rise in intraocular pressure. The same result was secured in perfusion experiments in which the tone of the capillaries was well maintained. When the capillaries were in a dilated condition the constrictor action of histamine on the arteries produced the predominant effect and a fall in intraocular pressure was observed. Examination of the anterior surface of the iris of the rabbit showed only a slight constriction of the vessels after histamine.

RELATION TO ANAPHYLAXIS. Anaphylaxis has been thoroughly discussed by Wells (1921) in these REVIEWS and in the recent edition of his book (1929), and by Longcope in his review on Anti-anaphylaxis and Desensitization (1923). It is unnecessary to do more here than to outline the possible relation of histamine to the subject.

Ludwig's pupils, Schmidt-Mulheim and Fano, observed as early as 1880 that a shock-like condition could be produced in animals by a single injection of a proteose-peptone mixture. This work was reviewed by Biedl and Kraus in 1909, who pointed out that there was a striking similarity between this peptone shock and the symptoms of anaphylaxis. They suggested that anaphylaxis might be a peptone intoxication. These observations paved the way for the association of the effects of histamine (and histamine-like substances) with anaphylactic shock. The first observations on this relationship were made by Dale and Laidlaw in 1910. In the guinea pig histamine produced a condition which corresponded in a suggestive manner to that caused by peptone and anaphylaxis in the same species. The following year the same authors noted that histamine produced in the dog and in the guinea pig a fall in rectal temperature. Pfeiffer and Mita (1910) had previously stated that such a decrease is characteristic of anaphylactic shock.

It had been observed by Dale and Laidlaw (1910) that one point of parallelism between the effects of histamine and those of peptone was lacking; namely, that peptone reduced the coagulability of the blood while histamine had no such effect. Biedl and Kraus (1912) however, found that in dogs, 3 mgm. of histamine would cause an immediate fall in blood pressure, retard the coagulation of the blood, and induce the phenomena generally characteristic of anaphylaxis. The increase in

clotting time of the blood in anaphylactic shock was much greater and of more constant occurrence than that of histamine shock. Kyes and Strauser (1926) reported that heparin inhibited in pigeons the production of anaphylactic shock. This inhibitory effect was not observed by Hyde (1927) in either anaphylactic or histamine shock in guinea-pigs.

The "anaphylotoxin" theory of anaphylaxis postulated the formation of a protein cleavage product of a highly poisonous nature. Vaughan had shown that products of protein hydrolysis might be highly toxic. This question was reviewed by him in 1913. He postulated that all true proteins contain a poisonous substance which can be split off by a mild alkaline hydrolysis. An incomplete protein, such as gelatine, does not contain the toxic group. This poison was considered to be identical with the anaphylactic poison which was responsible for the shock produced when a second injection of homologous protein was given to a sensitized animal. This condition could be simulated by a single injection of the protein poison, and the same effects were produced by poisons prepared from widely different proteins. A comparison with the results of histamine injection led Vaughan to believe that the amine might be a constituent of his protein poison. Friedberger had reported that under certain conditions toxic products might be produced *in vitro* by the action of antigen, antibody and complement. A similar reaction *in vivo* was thought to liberate "anaphylotoxin" and cause anaphylactic shock. In 1912 Friedberger and Moreschi investigated the possible relation between histamine and the "anaphylotoxin." There were many physiological similarities but chemical differences were found. Histamine was stable in acid and alkaline solution while "anaphylotoxin" was not. They concluded that "anaphylotoxin" was not histamine. As indicated elsewhere in this review the stability of histamine is greatly affected by the presence of other materials. While the "anaphylotoxin" theory seemed plausible and for some years received ample attention, it is now generally regarded as unsatisfactory.

A paper which had an important bearing on the later work on anaphylaxis was published by Dale in 1912. In it he reported that an isolated uterus from a sensitized guinea pig was extremely sensitive to small amounts of the antigen. The addition of a small quantity of the antigen to the uterus bath produced a maximal contraction, following which there was complete lack of sensitivity to the particular antigen. This work emphasized the importance of the reaction of smooth muscle and of the tissue cells in anaphylaxis and provided a foundation for later theories.

The similarity between the effects produced by histamine and those of anaphylactic shock was again pointed out by Dale and Laidlaw in 1919. The fact that histamine resembles in its effects a whole group of substances all of which act on the endothelium to produce changes of the same general type, was emphasized. In this paper the suggestion was made that the anaphylactic antibody was a precipitin, which reacted with the corresponding antigen to cause a change in the dispersion of colloid particles. The physical changes in endothelial cells would lead to relaxation of their tone and to abnormal permeability.

M. I. Smith (1920) investigated the possible relation of histamine to anaphylaxis and concluded from his observations that histamine shock was not identical with anaphylactic shock in rabbits and guinea pigs. Histamine produced no temperature changes in his animals while anaphylaxis did. Histamine did not alter the coagulability of the blood. Quinine augmented the susceptibility of sensitized animals to the foreign protein but it had no such effect during the production of histamine shock. We have already seen that a fall in temperature is often observed in several species of animals, including the guinea pig which Smith studied, after histamine.

The isolation of what appeared to be histamine from peptone and from hydrolyzed proteins led Abel and Kubota (1919) to state that the histamine content was responsible for the shock produced by peptone, and that histamine was the active material liberated in anaphylaxis. A criticism of this work was made by Hanke and Koessler in 1920. These authors were unable to find sufficient histamine in Witte's peptone to account for peptone intoxication and they claimed to have prepared, using aseptic precautions, a peptone which contained no histamine but which produced shock. Though the work of Abel and Kubota may be criticized on the grounds that aseptic technique was not used, it has been pointed out elsewhere in this review that the results of Koessler and Hanke were unsatisfactory in that the colour reaction on which their findings was based may have been inhibited by material present in their solutions.

Dale (1920) in his second Herter Lecture very thoroughly reviewed the subject of anaphylaxis and, as this paper is readily available, it is unnecessary to discuss it in detail here. Dale emphasized that anaphylaxis was an antigen-antibody reaction occurring in the tissue cells; a fact clearly demonstrated by the reaction of the isolated uterus. For this reaction blood was unnecessary. Dale discussed the two theories of anaphylaxis which were then current; the proteolytic explanation

based on the work of Vaughan and of Friedberger, and the physico-chemical theory which had been outlined by Dale and Laidlaw.

In 1921 Manwaring reiterated an explanation of anaphylaxis which he had originally advanced in 1910. He had demonstrated that dogs did not exhibit anaphylaxis if the liver had been previously removed from the circulation. The same was true for guinea pigs and he stated that, in these animals at least, the anaphylactic reaction was characterized by the explosive formation or liberation of smooth muscle relaxing substances by the hepatic parenchyma. No data on the chemistry of these substances were available.

In another paper Manwaring and his associates (1922) compared anaphylactic, peptone and histamine shock in dogs. In intact, sensitized animals each type of shock is characterized by a marked fall in blood pressure. Anaphylactic shock does not take place in dehepatized, sensitized dogs. That is, the liver is essential for the development of anaphylactic shock in dogs. Removing the liver reduces the severity of, but does not prevent, peptone shock. These investigators state that histamine shock is not influenced by hepatectomy. Manwaring concluded that canine anaphylactic, peptone and histamine shock were not physiologically identical reactions, at least in so far as their initial or fundamental physiological mechanisms were concerned. The secondary reactions due to low systemic blood pressure were presumably identical in the three types of shock. In the latter stages of each shock, the secondary reactions conceivably dominated the clinical picture. In this connection it is interesting that Ravdin (1929) has obtained evidence that the histamine content of dog liver is increased by obstruction of the biliary duct. He suggests that an accumulation of depressor substances may play a part in the shock-like condition sometimes observed in human patients after release of obstruction of the common bile duct.

Bally (1929) has compared the effects produced in rabbits by histamine, by peptone and by anaphylactic shock. The three conditions are very similar, except in one respect: in anaphylaxis there is a decrease in body temperature, which is not found in histamine and peptone shock. So far as blood pressure, blanching of the ear, effect on heart rate, coagulation time and engorgement of the right heart are concerned, there is similarity. It is very interesting that the point of difference between anaphylactic and histamine shock appears to vary in the different species.

Kellaway (1930) has found that the isolated uterus of a sensitized rat responds to the specific protein by contracting. In view of the

statements of the literature regarding the refractoriness of the rat to anaphylaxis Kellaway has emphasized that this response of the uterus is a regular occurrence. Histamine, however, in moderate doses relaxes the isolated rat uterus. The reaction of the uterus to histamine is not changed by the sensitization process, as the amine also relaxes the sensitized uterus. Hence Kellaway believes that histamine is not a factor in anaphylaxis in the rat. It is perhaps significant that small doses of histamine cause an increase in tone in the isolated rat uterus. Further observations on the change of the intact uterus of the rat during anaphylaxis would be valuable. It is interesting to note that the rat is relatively insensitive to anaphylaxis and to histamine. Voegtlin and Dyer (1924) found that the intravenous lethal dose of histamine for the white rat is 300 mgm. per kilogram.

In two recent papers Kendall (1930) and his associates have discussed the histamine hypothesis of anaphylaxis. They have compared the responses of the smooth muscle of the intestine of guinea pigs to protein and to histamine and find that the effect of histamine closely resembles anaphylaxis. Histamine differs from the action of the homologous protein on the sensitized intestine in several respects: 1, there is no sensitization phenomena connected with the histamine effect; 2, histamine produces an immediate effect in contrast to the lag found with the protein reaction; 3, to produce successive equal effects with the antigen progressively increased doses are required, while with histamine equal doses will give equivalent responses. These differences are explained if one supposes that the antigen reacts with the antibody in the cells to set free histamine or histamine-like substances. Such an explanation accounts for the lag in the protein effect—that is, the time required to liberate the histamine. One would expect that sensitization would not be required when histamine itself is added. The active substance is the same no matter what the protein. Kendall points out that the phenomenon of desensitization may be used as an argument in favour of the histamine theory. Desensitization may be regarded as a disappearance of the antibody from the tissues, and washing out of this restores the tissues to their normal unsensitized condition.

Our present knowledge of the mechanism of anaphylaxis and the rôle of histamine has been summarized by Dale (1929) in the third Croonian Lecture. An explanation which accounts for the similarity between anaphylaxis and histamine shock in various species, and also for the difference, is advanced. In anaphylactic shock, it is postulated, intracellular reaction between antigen and antibody causes cellular injury

which liberates histamine and other substances. The release of these other substances from the damaged cells may account for the signs which are observed in anaphylaxis but which are not seen in histamine shock. While this hypothesis lacks direct experimental evidence for its verification, it very satisfactorily fits the known facts. In this field, as in many others, rapid progress might be made if a sensitive method for the estimation of histamine in blood were available.

THE RELATION BETWEEN HISTAMINE AND THE ADRENALS. The first investigators to focus attention on the antagonism between epinephrine and histamine were Dale and Richards (1918). In perfused organs they showed that the addition of epinephrine to the perfusion fluid restored the tone of the vessels which could then be relaxed by histamine. They suggested that one function of epinephrine might be to maintain the tone of the capillaries. If histamine were liberated in the tissues the state of the capillaries would be determined, in part, by the relative amounts of histamine and epinephrine present.

Dale (1920) found that histamine, injected into an animal with a denervated pupil, caused the same effect as an injection of epinephrine, i.e., a dilatation of the pupil. It appeared probable that the injection of histamine had increased the output of epinephrine from the adrenal glands.

Kellaway and Cowell (1922) injected histamine intravenously into a non-etherized cat and observed transitory slowing of the heart, dilatation of the pupils, and sweating. In an animal with intact adrenals, an injection of histamine produced dilatation of the denervated pupil. In an animal from which the entire adrenals, or the medulla alone, had been removed, histamine caused no such dilatation. This was considered proof that histamine accelerated secretion from the medulla of the adrenal glands.

It was also noted by Kellaway and Cowell that in adrenalectomized animals, and in normal cats to which histamine had been given, a concentration of the blood as indicated by an increase in hemoglobin was produced. The symptoms following adrenalectomy suggested a poisoning by some toxin and, since histamine causes concentration of the blood, the possibility that it might be the toxic substance, was considered. The influence exerted by the cortex and by the medulla of the adrenals was studied by causing necrosis of the medulla with radium or by destroying the cortex by cautery. When histamine was not administered, loss of the cortex caused a prolonged and extensive concentration of blood, while loss of the medulla gave a transient and less

marked effect. The administration of histamine produced a prolonged, large increase in concentration when the medulla had been destroyed, and a transient, slight rise when the cortex had been eliminated. Without histamine, then, the loss of the cortex may be responsible for an increased blood concentration. Moreover, subcutaneous injections of epinephrine did not modify the increased concentration produced by adrenalectomy. There is no direct evidence that histamine is responsible for the concentration of the blood observed in adrenal insufficiency, even though in this condition there is certainly an increased sensitivity to the amine.

Lucas (1926) studied the changes in the blood of adrenalectomized dogs, which were being observed by Banting and Gairns. In confirmation of previous results, Lucas found that adrenalectomy caused an increased blood concentration and also increases in non-protein nitrogen, uric acid, chloride, calcium and phosphorus. Some of the changes were doubtless a direct result of blood concentration, while others from a consideration of autopsy findings, were thought to be due to kidney damage. These blood changes, and the symptoms seen in the animals, suggested to Banting and Gairns (1926) a possible rôle of histamine, and they compared the toxicity of histamine before and after adrenalectomy. Removal of the adrenals appeared to increase the sensitivity of the animal to histamine by about 30 times.

The increased response to histamine exhibited by adrenalectomized animals has also been observed by several others workers. Crivellari (1927) found that the lethal dose of histamine in normal rats was from 0.5 to 0.7 gram per kilogram; in adrenalectomized rats much smaller doses, 0.01 to 0.04 gram per kilogram, were required. In rats, then, adrenalectomy increased the sensitivity to histamine about 50 times. Marmorston-Gottesman and Gottesman, 1928) secured somewhat different results in rats. In their experiments adrenalectomy decreased the lethal dose from 1600 mgm. (five month old normal rat) to 80 to 100 mgm. This is an increase in sensitivity of about 20 times. These workers pointed out, however, that the susceptibility in normal animals varied greatly at different ages. The minimum lethal dose for the operated animals was the same whether given intravenously or intraperitoneally. They suggested that histamine be used to test adrenal insufficiency, since it is a stable compound which gives characteristic symptoms. Moreover the difference in minimum lethal dose for normal and operated animals is quite large. In continuation of this work, Perla and Marmorston-Gottesman (1929) investigated the effect of repeated

injections of epinephrine on the resistance of suprarenalectomized rats to histamine poisoning. The height of susceptibility of operated rats to histamine was reached at the end of the first week after the operation, after the cortical tissue had often, in part, regenerated. Repeated subcutaneous injection of 0.5 cc. of an epinephrine solution (1 mgm. per cc.), up to and including the day of injection of histamine, protected about one-half of the rats against a lethal dose. This protection was thought not to be the result of an immediate antagonism between histamine and epinephrine, since a single subcutaneous injection of epinephrine prior to injection of histamine only protected 2 to 8 per cent of the rats against a lethal dose. Wyman (1928) contended that an intraperitoneal injection of epinephrine provides sufficient in the blood stream to directly antagonize histamine. This was denied by Perla and Marmorston-Gottesman who found that a single intraperitoneal injection ten minutes before injection of histamine failed to protect. The latter group believe that the action of epinephrine is not due to a direct antagonism but to some indefinite indirect effect.

Scott (1928) felt that the increased susceptibility to histamine was due to lack of cortical tissue and suggested histamine as a test substance for cortical insufficiency. The relation of cortex and medulla to histamine sensitivity was further studied by Wyman (1928). In albino rats unilateral adrenalectomy was not found to increase the sensitivity to histamine. In these rats and in normal rats the lethal intraperitoneal dose was 100 mgm. per 100 grams of body weight while in completely adrenalectomized rats the lethal intraperitoneal dose was 7 to 10 mgm. per 100 grams of body weight. This dose caused death within an hour. The sensitivity of operated animals was measured at various periods after operation and was found to be constant up to at least five months. Adrenalectomized rats, killed with histamine, showed at autopsy considerable accessory cortical tissue. It is unlikely, therefore, that lack of cortical tissue was responsible for the increased susceptibility to histamine. As has been previously stated, Wyman found that an intraperitoneal injection of epinephrine protected a rat against histamine. He found that subcutaneous administration of epinephrine was relatively ineffective.

MacKay (1930) found that the effect of histamine on the salivary secretion of dogs and cats was greater and more regular after removal of the adrenal glands. Continuous intravenous injection of a dilute solution of epinephrine in these adrenalectomized animals diminished the effect of histamine. The suggestion was made, therefore, that the

failure to secure salivary secretion after histamine in certain experiments might be due to liberation of epinephrine.

It is apparent that there is conflicting evidence as to the mechanism of the antagonism between epinephrine and histamine, and also whether the medulla or the cortex or both are involved in protection against the action of the amine. Possibly further investigations of the action of the hormone of the suprarenal cortex may provide useful information on these problems.

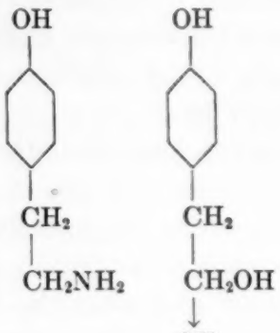
Various references to the epinephrine-histamine antagonism have been made in other sections of this review.

RISE OF BLOOD PRESSURE. The secondary rise of blood pressure attributable to liberation of epinephrine, which is observed under certain conditions after the injection of histamine, has been discussed in the previous section. Large doses of histamine may produce a rise of blood pressure in cats which are in histamine shock (Dale and Laidlaw, 1919; Meakins and Harington, 1922; Hogben, Schlapp and MacDonald, 1924). Alexiu (1929) has shown that this effect may also be observed after removal of the adrenals. The capillaries are apparently maximally dilated and further constriction of the arterioles is probably responsible for the rise of blood pressure. A rise in blood pressure in rabbits under ether and some other anesthetics, has been observed after histamine (Ackermann and Kutscher, 1910; Dale and Laidlaw, 1910; Feldberg, 1927). In guinea-pigs which have been anesthetized for some time the effect of histamine on bronchial musculature is diminished, and a rise in blood pressure may be clearly demonstrated after its intravenous administration (Dale and Laidlaw, 1910).

THE INACTIVATION OF HISTAMINE. A review of the work on the inactivation of histamine may be prefaced by a short consideration of the detoxification of an analogous amine, tyramine (parahydroxyphenylethylamine). Like histamine this base was isolated from ergot and from putrefied organic matter and it was shown to exert pharmacodynamic effects which are in certain respects similar to, but of less intensity than, those caused by epinephrine. Ewins and Laidlaw (1910) studied the fate of tyramine after oral administration to dogs, and after perfusion through isolated organs of rabbits and cats. From the urine of the dogs to which the amine had been given Ewins and Laidlaw isolated parahydroxylphenylacetic acid but in no case did they secure enough to account for more than 25 per cent of the amount of the amine which had been administered. Large amounts of tyramine were detoxified by perfusion in Ringer's solution through intact rabbit liver. The authors estimated

that a rabbit's liver might be able to inactivate about 120 mgm. of tyramine per hour. This would yield approximately 100 mgm. of hydroxyphenylacetic acid. Ewins and Laidlaw suggested that the detoxification of tyramine occurs in two stages:

(1) Replacement of the NH_2 group by OH



(2) Oxidation of the alcohol to the acid

There was no direct evidence for this intermediate step, but it seemed probable.

The work of Ewins and Laidlaw on tyramine laid the foundation for much of the subsequent investigation on the detoxification of histamine. Dale and Laidlaw (1911) endeavoured to inactivate histamine by adding it to fluid which was perfused through liver. They were never able to secure inactivation of more than 10 mgm. of the amine and they concluded that the identification of the end-product was hopeless under these circumstances.

Oehme in 1913 reported a comprehensive series of experiments on the inactivation of histamine. He first found that comparatively small (0.6 mgm. or less) doses were fatal to rabbits if given rapidly by the intravenous route. However, twenty times this amount could be given by slow infusion intravenously without harmful effects. Three possible explanations of the lack of effect of the slow infusions were considered by Oehme: 1, that histamine was eliminated (i.e., by the kidneys) while the infusion was being given; 2, that it was rendered innocuous by chemical or other means in the body; or 3, that the effective concentration of the base in the circulating fluid was never high enough at any

one time to cause shock. The first explanation, elimination, was discounted since during most of the experiments no urine was passed. When urine was removed by catheter no change in histamine content, before or after infusion, was observed. Direct examination of the blood by assay on the isolated uterus suggested that the histamine disappeared quickly from the circulating blood. Moreover, when blood from an animal, into which histamine had been infused, was hirudinized and circulated in another animal no harmful effects were produced. It seemed clear that physiologically large amounts of histamine quickly disappeared from the circulating blood. It was possible that it was held in stagnated blood in the liver, or that it had been changed chemically, perhaps by the liver. Oehme did not pursue this matter further, but he considered it probable that histamine was chemically altered in the body and that consequently the animal could dispose of large amounts if these were given slowly.

Eustis (1915), basing his hypothesis on the experiments of Ewins and Laidlaw, suggested that the liver was a detoxifying organ which could inactivate histamine. To test the correctness of the assumption he chose the turkey buzzard, *Cathartes aura*, which lives on carrion. This material is supposedly rich in histamine as a result of putrefaction. Eustis carried out an experiment in which the liver was aseptically removed immediately after killing a buzzard. The liver was minced aseptically, placed in sterile flasks, and a sterile solution of histamine in saline added. Before incubation toluene was added to the mixture. After 24 hours' incubation the contents of the flasks were tested for sterility and for histamine. Eustis found that 10 grams of liver pulp would inactivate 10 mgm. of histamine. He concluded that this detoxification was caused by a heat-labile system. This study was not continued.

The work which Ewins and Laidlaw carried out on tyramine was extended by Guggenheim and Löffler (1916), who investigated the changes produced in five amines by animal tissues. Four of these, parahydroxyphenylethylamine, phenylethylamine, indolethylamine, and isoamylethylamine, were inactivated in the same way as had been described by Ewins and Laidlaw, that is, by oxidation to the corresponding acid. A number of perfusion experiments were carried out with histamine, but in no case was an end-product isolated. They found no trace of iminazolacetic acid. Guggenheim and Löffler confirmed the results of Dale and Laidlaw and of Oehme. They found that 50 mgm. of the amine could be infused into a rabbit without harm, if the infusion were carried out sufficiently slowly. After such a slow infusion no histamine could

be detected in the urine, but when urine was secured continuously by catheter during the infusion some histamine was found. In samples of blood taken during a slow infusion no histamine could be detected. But if the histamine was given rapidly death ensued and histamine was present in the blood. To Guggenheim and Löffler these experiments indicated that the body possessed a mechanism for inactivating histamine as well as the other amines. Perfusion experiments were carried out with isolated rabbit liver and the results confirmed those of Dale and Laidlaw; only about 10 mgm. of the base could be inactivated in this manner. Mellanby's experiments, which have been described under "Absorption of histamine," showed that when the amine was introduced into the intestine of a cat a great deal of it disappeared. Enough entered the blood stream unchanged to lower the blood pressure, sometimes to fatal levels. We cannot conclude that all the histamine which disappeared was absorbed, and in light of recent work this seems unlikely.

The fate of absorbed histamine was further studied by Koessler and Hanke (1924). They, and others, had shown that histamine could be formed by intestinal micro-organisms and that it may be present in normal intestinal contents. The premises leading to this phase of their work are interesting and are quoted verbatim:

> For it deserves the meditation of the physiologist as well as of the clinician, how it is possible that such quantities of a highly poisonous substance could be harbored within the organism without producing symptoms of intoxication. There are several explanations which present themselves.
>
> 1. The histamine is destroyed within the intestinal lumen. The contents of the intestine, by means of their bacterial and cellular enzymes, might produce rapid changes in the structure and constitution of the amine of such nature that it is deprived of its toxicity. On this assumption the histamine found by us in the intestinal contents would represent only a transitional stage in protein metabolism and would not be an end-product.
>
> 2. Histamine is not absorbed. It might, for example, be so firmly adsorbed by the solid constituents of the intestinal contents that it is not free to pass through the intestinal wall.
>
> 3. Histamine is absorbed, but during absorption is at once detoxicated by the animal tissues (e.g., by the liver or by the cells of the intestinal wall itself).

The second question had already been answered by some of Mellanby's experiments and by Meakins and Harington; certainly some of the histamine which is placed in the intestine may be absorbed. Mellanby, and Meakins and Harington, secured marked depressions in blood pressure indicating that all the histamine was not detoxicated on passage through the intestinal wall. Answers to some of these ques-

tions were, therefore, available. Koessler and Hanke carried out two types of experiments. 1. Histamine was fed to dogs and guinea pigs. After definite intervals the histamine content of the material in the intestine, of the intestinal wall, and of the liver was determined. 2. Histamine was administered so that it would traverse various routes in the blood stream.

The amounts of histamine fed in these experiments were physiologically enormous. Guinea pigs were given 100 mgm. either in capsules or by stomach-tube, and a dog 500 mgm. without the production of harmful effects. A typical experiment is worth consideration. One hundred milligrams of histamine were fed to a guinea pig, which was killed two hours later. Histamine was found as follows: intestinal contents, 26.8 mgm.; intestinal wall, 4.7 mgm.; liver, 4.5 mgm. Koessler and Hanke do not state the amounts of histamine which might normally be present in these locations but they are unquestionably very small. Actually, then, approximately 73.2 mgm. of histamine disappeared from the intestine and only a small fraction of this was found in the intestinal wall and in the liver. More than minute amounts could not have reached the general circulation, for a guinea pig of the size used would have been killed within a few minutes by an intravenous dose of 0.5 mgm. Similar results were secured on dogs, and Koessler and Hanke drew the obvious conclusion that somewhere between the intestinal lumen and the blood stream a very large amount of histamine is inactivated. In other words, there is a detoxifying mechanism for histamine in the intestinal wall.

Koessler and Hanke confirmed the observations of others, that the liver does not inactivate histamine in appreciable amounts. On the other hand, they obtained some evidence that the liver may exert a mechanical effect. When histamine is injected so that it has to traverse any capillary network, whether in the liver or in a limb, its effect may be slightly reduced. The capillary network may be dilated and in the stagnated blood some histamine may be retained. The data bearing on this point accumulated by Koessler and Hanke can not be regarded as very convincing.

In 1925 Leiter reported on the metabolism of a number of iminazoles, but histamine was not included. He has stated that normally no iminazoles can be detected in the blood of the dog or of man. When an iminazole compound is injected, that particular compound can be found in the blood, but only for a short time after the injection. He concluded that the animal organism has a well-developed capacity for destroying the iminazole ring, especially when a side chain is attached.

Ivy and Javois (1924) gave doses varying from 0.5 to 0.75 gram of histamine by stomach-tube to Eck fistula dogs and found that such dogs were as tolerant as normal dogs. They concluded that the liver does not inactivate histamine.

The *in vitro* inactivation of histamine by formaldehyde has been discussed by A. I. Kendall and his associates (1927). The observation was made that if a small amount of neutral formaldehyde solution was added to a bath containing a uterus which had been caused to contract by histamine, the uterus was at once relaxed. Kendall suggested that this was due to the formation of a condensation of product of histamine and formaldehyde. It was concluded that the formaldehyde had no effect upon the uterus, because if the uterus was washed it would again contract with histamine. A number of compounds which might be thought to condense with histamine, including other aldehydes, were tried and it was found that carbohydrate aldoses and ketoses, acetone, dihydroxyacetone, trioxymethylene, chloral hydrate, methylglyoxal and polyglyoxal were all ineffective, while formaldehyde, acetaldehyde, paraldehyde, alcohol and acetophenone were effective. The effectiveness was inversely proportional to the size of the molecule.

In connection with some of the work on the inactivation of histamine, we have made a brief study of the effects of formaldehyde. It was found that results secured by assaying the histamine by the blood pressure method were different from those secured by the uterus method. When 300 times the amount of formaldehyde theoretically necessary to condense with the histamine is added to a solution of the amine there is, at room temperature, an almost immediate loss of approximately 50 per cent of the depressor activity of the histamine solution. This concentration of formaldehyde has no effect on the blood pressure. The balance of the histamine is inactivated if the histamine-formaldehyde solution is kept at room temperature aseptically for three weeks. This secondary loss is accelerated by raising the temperature to 37°C. With this concentration of formaldehyde, however, the histamine exerts no effect on the isolated uterus. Using much smaller amounts of formaldehyde, but still an excess over that required to unite with all the histamine, about one-half of the amine is inactivated as estimated on the uterus. The results are the same, under these conditions using the blood pressure method of assay. Experiments indicated that the larger amounts of formaldehyde were poisoning the uterus, completely obliterating the histamine effect. Formaldehyde, therefore, produces an immediate effect on a solution of histamine, causing a loss of approximately one-

half of the physiological activity, and a slow secondary reaction in which the balance of the activity is lost. It may be postulated that the immediate effect is due to the formation of the condensation product. The physiological activity lost in this immediate reaction is almost completely restored by boiling the mixture with hydrochloric acid.

Spinelli (1929) found that thyroidectomized guinea-pigs were less sensitive to histamine than normal ones.

Ellinger (1930) has recently stated that the physiological activity of a 1:10,000 solution of histamine is destroyed by irradiating the solution with ultra-violet energy.

In 1929, Best found that naturally occurring or added histamine was rendered physiologically inert when incubated in saline suspension under toluene with minced beef lung. The loss of activity could be prevented by heating the suspension at 90°C. for 4 minutes. It was concluded that the change in histamine was due to an enzyme system present in the lung. This investigation has been continued (Best and McHenry, 1930) and the name histaminase suggested for the enzyme system. A study of the distribution of histaminase in dog tissues shows that it is contained in relatively large amounts in the kidney and in the walls of the small and large intestines, in moderate amounts in blood, muscle, spleen, lung, adrenal and bladder, in doubtful quantity in the liver, and that it is apparently absent or present in very small quantities in the heart, skin and stomach wall. It is not excreted in the urine. Beef kidney is an excellent source of histaminase and the preparation of a stable dry powder from kidney has been described. Active extracts of this powder can be prepared. Histaminase has an optimum hydrogen ion range of 6.8 to 8.0, and an optimum temperature of approximately 38°C. It is inactivated at 60°C. Sodium and potassium ions have no effect on histaminase action, ammonia and sulphate are slightly inhibitory, while calcium, and citrate ions have a marked inhibitory effect. Potassium cyanide in concentration as low as 0.0005 molar prevents the action of histaminase. This and the fact that oxygen accelerates the reaction suggest that the histamine-histaminase reaction is oxidative. Histaminase has no effect on tyramine. It was concluded that histaminase may cause the rupture of the iminazole ring. These *in vitro* studies led to perfusion experiments in which histamine dissolved in blood or in saline was perfused through isolated kidneys, liver and other organs. The rate of inactivation of histamine by kidney tissue was much more rapid than that by the other organs studied.

In view of earlier investigations it was interesting that histamine was inactivated at a much greater rate by the kidney than by the liver. The relatively small amounts of histaminase in the liver suggest an explanation of the negative results of perfusion experiments secured by Dale and Laidlaw, Guggenheim and Löffler, Meakins and Harington, and Koessler and Hanke. The hypothesis advanced by Koessler and Hanke that histamine is inactivated in its passage through the intestinal wall is supported by the finding of large amounts of histaminase in this tissue. These results suggest that the body possesses a specific mechanism by which histamine can be rendered inactive.

The approximate minimal lethal doses in milligrams of histamine, administered intravenously, per kilogram of body weight for a few species are as follows: guinea-pig, 0.25–0.30; rabbit, 1–3; pigeon, 1–2; dog, 2.5–3; mouse, 250 and rat, 300. The results of a comprehensive study of the minimum lethal doses of histamine administered by different routes to several species have been reported by Schmidt and Stähelin (1929). An investigation of the histaminase content of the various organs of these species might provide interesting data, but more rapidly acting protective mechanisms, which have not as yet been thoroughly investigated in any species, are undoubtedly of greater significance in the production of this variation in susceptibility to intravenous or subcutaneous injections of the amine.

It is interesting to speculate on the fate of histamine which may be produced in the intestine by bacterial action, or may perhaps be contributed to the blood stream by the tissues where it has been shown to exist. If histamine is produced gradually in the small intestine it may be completely inactivated during passage through the intestinal wall. If histamine is present in the intestine in high concentration, as after artificial introduction of the amine, then it may pass through the mucosa so rapidly that the histaminase present cannot accomplish complete detoxification, and unaltered histamine may reach the blood stream. It must be remembered that the histaminase reaction is apparently slow and the rate is dependent on the relative concentrations of enzyme and amine. The histaminase content of the intestinal mucosa may be a protection against the small amounts of histamine which normally might be absorbed. If histamine enters the blood stream in fairly large amounts it causes dilatation of the capillaries and stagnation of the blood. In this trapped blood some histamine which is thus prevented from acting in other parts of the body may be held. Histamine stimulates the adrenals to secrete increased amounts of epinephrine which

counteracts many of the effects produced by the amine. Small amounts of histamine may be excreted in the urine. In various tissues, and particularly in the kidney, histamine may encounter histaminase which may change it in such a manner as to render it physiologically inactive.

PHYSIOLOGICAL SIGNIFICANCE OF HISTAMINE. It is very interesting to follow the gradual development of interest in histamine after the first report of its pharmacodynamic effects. Although considerable attention has been paid to the possibility that the action of the amine may be intimately related to the phenomena observed in anaphylactic shock and intestinal obstruction, the most productive single stimulus to investigation of the properties of histamine has been the similarity between secondary wound or traumatic shock and histamine shock. As we have attempted to show, many of these studies have yielded a great deal of valuable information in various phases of the biological sciences. There is, however, at present, no conclusive evidence that histamine is the causative agent in any pathological condition. While there is no indication that interest in this aspect of the subject is decreasing, the results of recent researches have drawn attention to the possibility that the amine may possess physiological significance.

The proof that histamine can be obtained by very simple methods of extraction from fresh normal tissues has provided an important link in the chain of evidence which may lead to the establishment of the amine's physiological rôle. A number of points provide interesting but indirect evidence in favour of this possibility. The presence of a histamine-like vasodilator material in human skin and the ease with which it can be released by mechanical stimulation or by nerve impulses, and the liberation of sufficient of this material in certain human subjects to cause flushing of the face, secretion of gastric juice, or lowering of blood pressure similar to that produced by the subcutaneous injection of histamine, are suggestive points. The fact that many physiological processes are affected in opposite directions by histamine and epinephrine together with the evidence that liberation of epinephrine from the adrenal glands is caused by injection of histamine suggest that these substances may be physiological antagonists, while the demonstration of what appears to be a specific enzyme system for the inactivation of the amine indicates that a slower acting protective mechanism may also be available. The presence of relatively large amounts of histamine in the lungs of certain species is undoubtedly of physiological significance, but attempts to determine what this significance is have not yet succeeded. The evidence that the production of local effects seems to be the most im-

portant function of the histamine-like substance suggests that it is only liberated in quantities which are chemically very minute. If this is true, it will be very difficult to establish whether or not the amount of the substance in a tissue varies under physiological conditions. The importance of this question and the inherent difficulties of its investigation are certain to attract scientific workers.

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INNERVATION OF THE STOMACH

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Considerable attention has been directed toward the study of the motility of the stomach and its regulation by the extrinsic nerves, but in spite of many investigations, our knowledge is still very incomplete. This is probably due to the complicated nature of the peripheral nervous mechanism and, further, our ignorance of the physiology of plain muscle.

Critisicm may be directed against many experiments, as investigators have not paid strict attention to the use of anesthetic and preparation of the animal. It is not fully realized that the stomach is an organ extremely sensitive to shock, indeed, as Hughson (70) has pointed out, even simple laparotomy will cause inhibition of movements and loss of tone, which may last unless precautions are taken, for a considerable period.

The introduction by Cannon (29) of the x-ray technique for the study of the position, size and movements of the stomach made it possible to make qualitative observations and to investigate the factors regulating motility. For quantitative records, however, recourse must still be made to direct methods and the use of decerebrate and decapitate preparations allows experiments to be made without the use of anesthetics. These preparations, while offering considerable advantages, are not ideal, as the actual operation causes considerable shock to the stomach. Further, the recent work of Beattie, Brow and Long (11) suggests that decerebrations made at certain levels may injure nuclei of the hypothalamus, thus interfering with the extrinsic nerve supply.

While the use of isolated vagus and sympathetic smooth muscle preparations possesses obvious disadvantages, namely, loss of blood supply, anoxemia, etc., it nevertheless affords the only means of making a quantitative study of the effect of stimulating the vagus and sympathetic nerves and following accurately the response of the muscles to different strengths and frequencies of stimulation.

The excellent monograph of Cannon (30) published in 1911 deals with the mechanical factors of digestion, while Alvarez (2) in his recent book

considers the information more particularly from the viewpoint of diagnostic and therapeutic problems. Evans (57) in 1926 reviewed fully the physiology of smooth muscle, with particular reference to the chemical changes accompanying contraction, and the scope of this article is therefore restricted to a review of the nerve supply, movements of the stomach, and the function of the vagus and the sympathetic nerves with relation to the motility of the organ.

The conflicting evidence on the peripheral function of the vagus and sympathetic nerves prohibits in our opinion any detailed treatment in the present article of the sensory and reflex phenomena of the stomach.

THE STRUCTURE OF THE MUSCLE. On examination of the human stomach and the stomachs of animals other than ruminants, the regions are found to vary in size and arrangement, but the muscular layers are relatively constant (24). On inspection three layers may be distinguished in the muscular coat: external, middle and internal.

The external layer, consisting of longitudinal fibres which are continuous with the corresponding fibres of the esophagus and duodenum, is best developed over the greater and lesser curvatures; between these regions the fibres are thin and scattered.

The middle layer is made up of circularly arranged muscle fibres, which in the rabbit, cat and dog form the only complete layer of the muscular coat. The fibres are continuous with those of the corresponding layer of the esophagus and duodenum. They radiate outwards and upwards in the fundus from the thickened band of circular fibres forming the cardiac sphincter at the junction of the esophagus and stomach. The circular muscle is thickened over the pyloric antrum, especially in the dog. The prepyloric sphincter situated at the junction of the body of the stomach and the pyloric antrum has been described by Cathcart (40).

The internal layer is represented in the cat and dog by a muscular band on each side of the lesser curvature, passing from the fibres of the cardiac sphincter to the circular coats of the pyloric antrum. Forssell (60) and Jefferson (73) have described similar fibres in the human stomach as the lower segment sling forming the canalis gastricus along the lesser curvature. Seber (136) and others have demonstrated the horseshoe sling in the cat and dog. The fibres which act as the sling are intimately connected throughout their course with the middle circular coat; below, with the fibres of the pyloric antrum; above, with the cardiac sphincter, where they join the band of circular fibres. The bundles pass down on each side of the lesser curvature to terminate as

a fan-like insertion, near the thickened muscle of the antrum. Throughout its whole course the sling sends out fibres from its outer edge, which turn outward to join the circular layer. The inner edges are very distinct, especially in the upper two-thirds of their course; fibres only leave to communicate with the other layers in the lower third.

The intimate relationship between the sling fibres and the circular muscle at the gastro-esophageal opening will be observed. It is difficult from the anatomical point of view to state whether the muscle of the cardia should be considered as part of the middle or of the internal layer of the muscular coat. It is probable that the sphincter muscle is functionally part of the sling system (111). The two muscle bands on the lesser curvature, forming the internal oblique layer of the stomach, are connected above to the circular fibres of the cardia; below, they are attached to the pyloric antrum, and throughout their course the fibres communicate with the circular layer. This communication is important, especially in the fundus region where the circular fibres radiate outwards and upwards.

Barclay (8) draws attention to remarkable alterations in the shape and position of the stomach due to various factors. Instancing the investigations of Jefferson (73) on the human stomach, he suggests that the internal oblique fibres may function as a sling, the varying appearance of the stomach being due to the state of contraction of these fibres. It is probable that the fibres of the cardia are part of the sling mechanism. The "tonus" of the sling fibres would then not only determine the shape and position of the stomach, but would control and regulate the movements of the cardia.

Abdominal distribution of the vagus nerves. Many workers have described the abdominal distribution of the vagus nerves, but the work of most interest is found in papers by Swan (139) 1834, Bourgery (18) 1844, Kollmann (85) 1860, Ducceschi (51) 1905, Perman (127) 1916, Latarjet (92) 1921, Brandt (19) 1920, and McCrea (103) 1924.

McCrea reviews the earlier work on the abdominal distribution of the vagus nerves. He subdivides his account of the nerves in man into three headings: 1, the structure of the esophageal plexus, 2, the branches and distribution of the anterior vagal trunk, and 3, the branches and distribution of the posterior vagal trunk.

Both vagi having passed posteriorly to the lung roots incline anteriorly and medially and proceed to divide—the right usually into three branches, sometimes four, the left almost invariably into three. These branches are then arranged about the lower third of the esophagus: two branches

of the left nerve pass to the anterior aspect of the esophagus and there unite with one small branch of the right nerve. Together these nerves form a very simple plexus on the anterior surface of the esophagus, and from this one or two stems pass through the esophageal opening of the diaphragm forming the anterior vagal trunk.

Two or three divisions of the right vagus and one of the left pass to the posterior aspect of the esophagus and there form a plexus, from which usually arises a single trunk, the posterior vagal trunk, passing through the esophageal opening of the diaphragm.

McCrea (103) confirms the general description of the plexus given by Swan (139), Bourgery (18), and Kollmann (85), and points out that the plexus possesses a most constant structural basis and that each vagus supplies fibres to both the anterior and posterior surfaces of the stomach. The structure of the esophageal plexus in the cat and dog is essentially similar to that in man (Kollmann, 85; McCrea, 103; Ellenberger and Baum, 55).

The anterior vagal trunk having passed through the esophageal opening as one or two stems supplies fine twigs to the lower end of the esophagus and to the cardia. The main branches may be divided into two groups, a group lying to the right and on an anterior plane, and a group to the left on a deeper plane.

The main branches of distribution of the left group associate themselves with the anterior branches of the left gastric artery and supply the region of the anterior surface of the cardia, fundus and proximal portion of the body of the stomach. The right group gives origin to three main branches. The first branch is frequently duplicated, and its terminal twigs are divided into two series—a proximal, which can be traced into the porta hepatis, and a distal from which twigs pass *a*, to the pylorus and first stage of the duodenum; *b*, to run with the right gastric artery to the region of the pyloric canal; *c*, to run deep to the pylorus and first stage of the duodenum with the gastro-duodenal artery, and so toward the head of the pancreas and second stage of the duodenum; *d*, to run on the wall of the hepatic artery, and *e*, to communicate with sympathetic twigs passing to the gall bladder. The second branch passes down between the layers of the lesser omentum parallel to the lesser curvature and is distributed to the anterior surface of the pyloric antrum and body of the stomach in the neighbourhood of the incisura angularis, but does not reach the pyloric canal. The third branch follows the lesser curvature close to the line of attachment of the lesser omentum and may be traced to the incisura angularis. The anterior vagal trunk gives off

one, sometimes two or three, branches to the liver which cross in the lesser omentum. Swan (139) and Kollmann (85) state that these branches usually communicate with sympathetic fibres. Swan (139) states that twigs descend from this anastomosis to the pylorus and first stage of the duodenum. Latarjet (92) affirms that the pylorus receives its entire nerve supply from sympathetic fibres accompanying the hepatic artery.

The distribution of the nerve is, in the main, similar in the cat, dog and rabbit (Kollmann, 85; Ellenberger and Baum, 55; Latarjet, 92; McCrea, 103).

The posterior vagal trunk, after supplying the lower esophagus and cardia, passes through the esophageal opening and divides into two main branches. The major portion passes proximally with the stem of the left gastric artery and supplies fibres to both right and left coeliac ganglia. Other fibres may be traced directly on to the splenic artery and to the pancreas and some anastomose with sympathetic fibres which run with the superior mesenteric artery and hepatic artery and to the renal and suprarenal plexuses. The fibre for the stomach passes for a short distance along the lesser curvature and sends twigs to communicate with branches of the anterior vagal trunk. A branch supplies the posterior aspect of the proximal portion of the body and gives off branches to the posterior surface of the body and pyloric antrum, these branches at their termination associating themselves with branches of the left gastric artery. The most distal is distributed in the neighbourhood of the incisura angularis, but does not reach the pyloric canal. The description given by McCrea confirms in general the findings of Perman (127), Latarjet (92), and Kollmann (85).

Distribution of sympathetic fibres to the stomach. The sympathetic fibres to the stomach, according to McCrea (103), are entirely derived from the coeliac plexus.

The fibres are grouped as follows:

a. Fibres from the coeliac plexus pass with the left inferior phrenic artery and so to the stomach; they wind about the anterior aspect of the lower extremity of the esophagus from left to right, communicate with branches of the anterior vagal trunk and are distributed to the region of the cardia and fundus.

b. Fibres pass with the left gastric artery from the coeliac plexus, and may be divided into three groups:

1. Passing with the esophageal and superior branches of this artery to the cardia and proximal part of the body of the stomach; reinforce-

ments are received from the fibres with the right inferior phrenic artery. These twigs may be traced to the cardia and to communicate with branches of the anterior and posterior vagal trunks.

2. Fibres which pass with the main stem of the artery along the lesser curvature to supply both surfaces of the body of the stomach and pyloric antrum; one branch lying closely applied to the lesser curvature is of considerable size.

3. Fibres passing across between the layers of the lesser omentum towards the porta hepatis, communicating with the hepatic branches of the anterior vagal trunk in the majority of instances.

c. Fibres pass from the coeliac plexus along the hepatic artery; these form several large nerves and are distributed with its branches; they reach the pyloric region of the stomach with the right gastric and right gastro-epiploic arteries.

Group *b* is much the most prominent and supplies the major portion of the stomach.

Rasmussen and Duncan (131) described vagus fibres in the splanchnic nerve of the cat, which they state are small and functionally insignificant. Duncan (54) also observed vagus fibres in the splanchnic nerve as a result of examining the nerve in cats, dogs and rabbits after section of the right vagus.

The work of Beattie, Brow and Long (11) on the existence of nerve tracts connecting the hypothalamus with spinal sympathetic centre has made it clear that the original conception of Langley (90) and Gaskell (62) of the anatomy of the sympathetic system is incomplete. Beattie, Brow and Long have shown that extra systoles induced by chloroform are removed by section of the hypothalamus at a plane between the anterior edge of the colliculi and the posterior edge of the pituitary fossa. Experimental lesions involving certain nuclei of the posterior part of the hypothalamus are followed by descending degeneration in the spinal cord. These fibres are shown to play an important part in the control of the bulbar and spinal sympathetic nuclei. This conception receives confirmation from experiments quoted by these authors which demonstrate that certain aspects of carbohydrate metabolism, temperature regulation, pilomotor and ocular phenomena, pressor effects, etc., depend on the function of nuclei existing in the hypothalamus and other parts of the brain stem.

THE ENTERIC PLEXUSES. A detailed description and good bibliography of the anatomy of the enteric plexuses of the gut may be obtained in C. J. Hill's paper (66) or a more general account from Maxi-

mow (101). The extrinsic fibres are stated to enter the gut wall along the larger blood vessels, and comprise *a*, vagal fibres of uniform size in the form of bundles, which have little or no connection with the blood vessels and pass directly to the enteric plexuses; *b*, sympathetic fibres exhibiting various properties which are more closely bound up with the vessel walls. The sympathetic fibres penetrate the gut wall and form at the outer surface of the circular muscle coat a fine plexus, which is well marked in the stomach.

Dogiel (48) in 1899 described two types of cells in the enteric plexuses: *a*, type I: multipolar cells present only in the myenteric plexus, and *b*, type II: cells of three different varieties, multipolar, bipolar and unipolar, with long dendrites present in both myenteric and submucous plexuses. Hill confirms Dogiel's classification of the enteric neurones into types, and does not support the findings of Kuntz (86), Carpenter (39), and Johnson (74), who consider all possible gradations exist. The first type occurs exclusively in the myenteric plexus. It is a multipolar cell with short dendrites which end on cells of type II, it being, as Hill points out, impossible to accept Johnson's (74) view as to the non-existence of synapses between the local neurones. The axon enters a fibre bundle as a fine non-medullated fibre and can be traced for a considerable distance through the neighbouring ganglia and fibre tracts, and is supposed to form connections with cells of the second type in other ganglia. These cells are therefore of an associative nature. The cells of the second type are far more numerous and show great variations in their form. The dendrites end in relation to the nerve cells of the first and second type. The axon enters a fibre bundle which divides, and the branches pursue separate courses, ending in connection with individual muscle cells. A third type of cell—"interstitial"—of microglial nature is found scattered in the submucosa and in the interior of the villi.

The plexuses are essentially composed of cell bodies and processes of the cell bodies which are in synaptic relation to each other. Dogiel (48), Kuntz (87), Carpenter (39), and Johnson (74) are in agreement concerning the endings of the vagal fibres in the form of pericellular arborization. Hill, however, supports the suggestion of Müller (119) and Kuntz (87) that the neurones of the enteric plexus are vagal, and states that the neurones of type II receive the pericellular arborizations of the vagal fibres, and that these constitute the second neurones in the vagal path and are therefore motor in function. Gaskell (62) also held that the vagal nerve fibres which traverse many ganglia and give off collaterals

must terminate round cells of type II. He pointed out that the innervation of the gut is of the same character as that of other organs innervated by the involuntary nervous system: the vagal fibres are connector fibres which connect with many cells by means of collaterals, and these cells are the motor cells to that part of the gut musculature which belongs to the vagus system.

The more diffuse endings described are, according to Hill, derived from dendrites of local ganglion cells, and are found on neurones of both types. The plexuses of Auerbach and Meissner are connected by bundles which run at regular intervals through the circular muscle. The fibres from the submucous plexus pass up to the muscularis mucosae and the smooth muscle in the villi to terminate in relation to the muscle cells.

The fibres from the sympathetic inflow and the axones of the motor cells of the myenteric plexus form an intramuscular plexus, the fibres of which run parallel to the muscle cells. The two sets of fibres are indistinguishable from each other and probably end round or actually in the muscle cell. Hill's observations on the motor nerve endings in smooth muscle are in general agreement with the investigation of Agababow (1), Boeke (16), (17) and Lawrentjew (95). The fibres of the intramuscular plexus on approaching the muscle cells break up into a number of fine fibres which form a complicated network extending over the surface of the cell. Delicate fibrillae which penetrate the substance of the muscle cell arise from small varicosities on the fibrillae.

Fibres of a delicate plexus in the subserous coat, formed from some of the fibres of the sympathetic inflow, appear to end freely in the connective tissue and are suggested to be sensory in function. Hill's preparations have not enabled her to observe the actual endings on the muscle cells, but many fibres have been traced from their origin in Meissner's plexus into the smooth muscle of the villi. These fibres have also been observed by Kuntz (88).

THE GRADIENT OF ACTIVITY. Alvarez (5), (6) has drawn attention to a gradient of tone, rhythm and activity of the stomach, and suggests that peristaltic waves have their origin in the most highly rhythmic sensitive area. He speaks of the region on the lesser curvature next to the cardia described by Keith (77) as the pace maker of the stomach. The local differences in the shape and depth of the peristaltic wave correspond to the regional peculiarities in tone and amplitude of contraction of the muscle fibres through which it must pass. At the upper end of the stomach the waves have a low amplitude and a high frequency, while, at the lower, they will have a large amplitude and a low frequency,

Alvarez (3) found from a study of excised strips of the stomachs of rabbits, cats, dogs and man that each region has a characteristic type of movement: he states that the rate of contraction of the strip varies inversely as the distance from the cardia. Brown and McSwiney (24) demonstrated that the reaction of the strips of the stomach to drugs shows that the musculature of the various parts of the organ possesses very different properties. The excitatory effects of pilocarpine are expressed as an initiation or augmentation of rhythmic movement which may or may not be associated with a permanent shortening of the muscle fibre. It is possible thereby to divide the stomach functionally into two parts:

a. The upper body and fundus which show a permanent shortening with little rhythmic augmentation.

b. The lower body and antrum which react with great augmentation of movement, and with little or no change in the permanent length of the muscle fibre.

These fundamental differences in reaction are further displayed by the use of the inhibitory drugs, atropine and adrenaline, since they exert little or no action upon the length of the fibre in lower regions, but alter profoundly that of the upper regions.

Action of pilocarpine

REGION	RISE IN BASE LINE	RHYTHMIC MOVEMENT
Cardia	+++	0
Fundus	+++	+
Upper body	++	+
Lower body	+	++
Pyloric antrum	0	+++

The power of postural adaptation is most clearly shown in the fundic region and decreases progressively to the pylorus. The motor power of the stomach varies inversely with the possession of this capacity of postural adaptation: thus, from the pyloric region which exhibits the most powerful contractions, postural changes cannot be elicited. Each region has a characteristic type of contraction which resembles closely the movements described in the intact stomach. A gradient of frequency of contraction could not be established by these observers.

THE MOVEMENTS OF THE STOMACH. The investigation of the normal gastric movements in animals and man may be grouped into one of two schools.

The first school holds that a typical gastric movement consists of two distinct phases:

a. A peristaltic wave which involves the body of the stomach and pre-antral region.

b. A contraction of the musculature of the pyloric antrum as a whole.

The second school, founded by Cannon (29), suggests that the peristaltic wave sweeps from its origin on the body of the stomach to the pylorus and does not partition the stomach.

Hoffmeister and Schutz (67) studied the movements of the isolated stomach of the dog. They state that the typical movements consist of a sequence of contractions, each of which is divided into two phases: first, the pre-antral contraction followed by contraction of the "sphincter antri pylorici." The investigations of Auer (7) on the rabbit supported the findings of Hoffmeister and Schutz. Bechterew and Mislawski (12) also found that the pyloric and cardiac regions of the stomach apparently function independently. Ducceschi (52) by the use of balloons demonstrated that the pyloric antrum had its own distinct systole and diastole. Groedel (63) confirmed on man the findings of previous observers. Holzknecht (68) and Hurst (71) support this school.

Cannon (29) was the first investigator to use x-rays in the study of the normal movements of the cat's stomach. He observed that within five minutes of the ingestion of food a slight annular contraction appeared near the duodenal end of the antrum, which moved peristaltically to the pylorus; this was followed by several waves recurring at regular intervals. Two or three minutes after the first movement was seen very slight constrictions, most marked on the greater curvature, appeared near the middle of the stomach and coursed slowly distally. Over the pars pylorica the waves deepened, and as digestion proceeded grew stronger, but they did not entirely divide the cavity until the stomach was nearly empty. The waves occurred at intervals of 10 seconds, and the passage of a wave across the stomach occupied approximately 36 seconds. The stomach emptied in 7 hours after a meal of 15 grams of bread.

He observed that as digestion proceeded the antrum appeared to elongate, but that its change of form, as compared with that of the remainder of the stomach, was slight. As the process continued and the stomach contracted on its contents, the mid-portion of the stomach took on a tubular shape; this tube had at the one end the tonically contracting fundus and at the other the active antrum. The fundus

forced the gastric contents into this tube, and they were then propelled into the antrum by a series of peristaltic waves which reached the pylorus. The pylorus did not open before each peristaltic wave.

He agreed with the previous investigations of Rossbach (134) who made direct observations of the gastric movements in dogs. Roux and Balthahazard (135), almost simultaneously with Cannon, examined the movements of the stomach of the dog and man by means of x-rays. As a result of their investigation they divide the stomach into two functional regions: *a*, superior and larger, the fundic reservoir, and *b*, a pyloric region, the motor portion of the organ which shows periodic peristaltic movements. Magnus (99) described the movements of the cat and confirmed the observations of Cannon. Kaestle, Rider and Rosenthal (75) using serial roentgenograms examined the movements of the normal human stomach, and concluded that the peristaltic waves commenced at the incisura cardiaca and travelled with increasing depth toward the pylorus. The old antrum disappears, and a new antrum is developed from the wall of the body of the stomach. The new antrum passes pyloruswards and ultimately takes the place of the old antrum, while another antrum begins to form. The appearance observed in the pars pylorica is stated to be due to an exaggeration of the peristaltic wave as it passes over this region. Cannon (30) quoted the work of Kaestle, Rider and Rosenthal (75) in support of his earlier findings.

Cole (41) endeavoured to correlate the views of observers of these two schools, and applied the term "individual peristaltic contractions" to the wave of contraction which passes over the stomach, and that of "gastric cycle" to the formation and duration of an individual antrum. He has classified the different types of peristalsis: *a*, one cycle type; *b*, one and a half cycle type; *c*, two cycle type; *d*, three and four cycle type, and *e*, type described by French authors as the "choreic type."

Wheelon and Thomas (149), from a study of movements in dogs with the abdomen open, concluded that the continuous type of peristaltic wave was normal for the moderately filled dog's stomach.

McCrea, McSwiney, Morison and Stopford (108) reinvestigated the subject and studied the movements in the rabbit, cat, dog and human, by direct observation and x-rays. They found that the movements in man were definitely related to the shape of the stomach. In the "J-shaped" stomach, peristaltic waves were observed progressing towards the pylorus and ending in the region of the pyloric antrum, a well formed spherical part of the stomach, which exhibited rhythmic concentric contractions and was often completely separated from the body of the stomach.

If the pyloric antrum is completely separated from the body of the stomach, then with each concentric contraction the antrum empties into the duodenum. Peristaltic waves are not seen passing over this type of antrum. If the pyloric antrum is not completely separated from the corpus, then the contents may not pass into the duodenum, but are returned into the body of the stomach. In this type, fine waves may be seen on the antrum.

In the "Steerhorn" stomach, situated high up and lying obliquely across the abdomen, the pyloric antrum was small and tubular in form rather than spherical and was never completely separated from the corpus. Concentric contractions were not so marked, and peristaltic waves could be seen passing right up to the pylorus.

The form of movement known as the two-phase type was most frequently found in the stomach of man, dog and rabbit, while the one-phase type, or a modification of this, was normally observed in the stomach of the cat. Either form, however, may on occasion be found in the stomach of man, cat and the rabbit.

These observations agree with the previous work of Cannon and Magnus, as their description is based on experiments made on cats. Further, the appearance of a separate antrum or even a double antrum as seen by Kaestle, Rider and Rosenthal may be explained by the fact that the constrictions in the pyloric region may be so deep as to obliterate completely the lumen.

There is therefore little difference in opinion, if it be realized that the movements of the stomach vary from animal to animal and from man to man according to the structure and position of the organ.

Klein (83) describes experiments with dogs which were slightly etherized. He states that there is no such thing as a single normal type of peristaltic wave. The two main types were observed but variations were common. The variations which occur within these types of waves are:

a. The wave passes down over the body reaching the angle and ending there; no antral activity follows.

b. Antral waves may start at an angle and pass to the pylorus as independent waves and not follow visible waves coming down from above.

c. Retrograde waves may start at the pylorus and run back over the antrum.

Alvarez (4) has recorded the movements by taking motion pictures, and his records offer an apparent solution to the problem. The records

obtained from dogs show the systolic type of contraction in the circular fibres of the last centimetre or two of the stomach. The point at which the waves change into systole varies in different animals and from time to time in the same animals, and the dissociation between the activities of the fundus and pars pylorica was established.

DENERVATION OF THE STOMACH. A considerable number of observations have been made on the experimental resection of the extrinsic nerves of the stomach, and the literature is extensive. The immediate results of nerve section have been carefully noted by several workers (Klee, 79, 81, 82, and others), but these effects cannot be regarded as of considerable physiological importance, for they are of brief duration and indistinguishable from the effects of shock and anesthesia. The more remote effects are of greater interest and importance. The method employed to record the condition of the stomach is important, and x-ray examinations are more reliable than the observations which have been made by gastric or duodenal fistula.

The nerves of the human stomach have received much attention, and various operations have been devised for division of the vagus and splanchnic nerves in certain disorders and diseases of the stomach. For the literature on this subject, reference should be made to the paper by McCrea (104), which provides an excellent bibliography. The experimental results obtained by the chief workers on animals are set forth in tables 1, 2 and 3.

Ducceschi (53) found that while on section of the vagi there is no very striking modification in the form and course of the normal movements of the stomach, excision of the coeliac plexus sets up movements with a periodic course, which arise from the grouping and combination of rhythmical movements, peristaltic waves, and oscillations of tonus, constituted into successive groups of fairly regular form. Subsequent division of the vagi modifies the groups, without, however, suppressing them.

From the examination of results obtained by these workers, certain deductions may be made:

1. That section of one vagus or one splanchnic is without effect.
2. That vagal section results in dilatation, diminution of tonus, slow and weakened peristalsis and delayed emptying.
3. That splanchnic section leads to the opposite effect and results in acceleration of function.
4. That section of all nerves gives similar but not so well marked symptoms as vagal section alone.

TABLE 1

Vagotomy

AUTHOR	ANIMAL	PERIOD OBSERVED	DILATATION	CONTRACTION		EMPTYING TIME	
				Depth	Interval	Initial	Total
		weeks					
Cannon (30, 31)	Cat	2–4	After 3 days normal			Delayed	
Carlson (35)*	Dog	2–12	−	+?	+		
Nieden (121)			+?	No change	No change	Slight delay	
Koennecke (84)	Dog, cat	45	+	Decreased	+	Delayed	
Watanabe (147)	Dog	2	+	Decreased	Irregular	Early slight delay	Delayed
Stierlin (138)	Dog	2–10	+	Decreased	−	Slight delay	
Litthauer (97)	Dog	3	+	Decreased	Decreased	No change	Delayed
Litchenbelt (96)	Rabbit, cat, dog		9 of 22 −	+	−		
McCrea, McSwiney and Stopford (110)	Cat, dog	Up to 80	None	+	Decreased or no change	Marked decrease	Slight decrease or within normal limits

* Carlson only studied hunger contractions.

TABLE 2

Splanchnotomy

AUTHOR	ANIMAL	PERIOD OBSERVED	DILATATION	CONTRACTION		EMPTYING TIME	
				Height	Depth	Initial	Total
		weeks					
Cannon (30, 31)	Cat	4	Nil	Normal	Normal	Very little change	
Carlson (35)	Dog	8	–	–	Decreased		
Koennecke (84)	Dog, cat	18	–	+	Decreased	Hastened	
Nieden (121)			Nil	No change	No change	No delay	
Watanabe (147)	Dog	4	Slight –	Slight increase		Almost normal	Hastened
Stierlin (138)	Dog	2–10	–	Good		Hastened	
Litthauer (97)	Dog	3	–	–		No change	Hastened
McCrea, McSwiney and Stopford (110, 104)	Cat, dog	28	Nil	+	No change	Marked decrease	Decreased

TABLE 3

Denervation

AUTHOR	ANIMAL	DILATATION	CONTRACTION		EMPTYING TIME	
			Depth	Interval	Initial	Total
Cannon (30, 31)	Cat	Very small stomach	No change	No change	Slight delay	
Carlson (35)	Dog	–	Increased	Increased		
Nieden (121)		Slight			Slight delay	
Latarjet (92)	Dog	+	Decreased	Increased	Delayed	
Watanabe (147)	Dog	+	Decreased	Increased	Delayed	
McCrea, McSwiney and Stopford (110, 104)	Cat, dog	None detected	Increased	No change	Marked decrease	Normal

According to McCrea, McSwiney and Stopford (110), the effect of nerve section is divided into two stages—an initial inhibition and paresis, followed after an interval by a return of normal function. Nerve section produces only one permanent symptom, i.e., a decrease in initial emptying time.

It is difficult to provide adequate reasons for this divergency of results. The pylorospasm and cardiospasm described by Klee (79, 81, 82), Exner and Schwarzmann (58) as immediate on vagal section are purely temporary and probably due to irritation of the nerve section. The retention is then probably due to early gastric paresis aided by reflex irritation of the remaining fibres. Some investigators may have paid too little attention to normal variations in peristalsis and emptying time, and these may be considerable. The period of post-operative examination has, in many instances, been too short, as Hughson (70) has pointed out that any abdominal operation, even a simple laparotomy, delays the emptying of the stomach, and this effect may persist for some time. Koennecke (84), however, who states that there is a definite delay in emptying, carried out his investigations over a period of eleven months.

It appears clear therefore that the immediate effects of vagotomy, splanchnotomy and denervation of the stomach are similar, namely, retardation of function. After a period the peripheral intrinsic nervous mechanism assumes control of the denervated stomach, and only one permanent symptom of importance, i.e., a decrease in initial emptying time, is observed. It would appear that both vagus and splanchnic nerves are together necessary for the normal regulation of the stomach. The cutting out of one system of regulators gradually results in a diminution and almost complete disappearance of control by the remaining system, the final result approaching but not attaining the effects of section of both.

THE EFFECT OF STIMULATION OF THE VAGUS NERVES. Many investigators state that the action of the vagus nerves on the stomach is predominantly motor, but there is good evidence to show that stimulation of the nerve may cause inhibition of movement and relaxation of the muscle. It has been accepted that either the right or left vagus through the esophageal plexus may innervate the whole stomach (Morat, 118; Ducceschi, 51; McCrea, 103), but Openchowski (124), Bechterew and Mislawski (13), Batelli (9), and May (102) have noted that stimulation of the left vagus nerve may cause a greater effect than stimulation of the right nerve.

The action of the vagus nerve may be considered, first, on the cardia and the fundic region, secondly, on the body of the stomach, and thirdly, on the pyloric region. These regions differ structurally from one another, and the response of each region may be shown to be characteristic.

Cardia and fundic region. In 1889 Openchowski (124) described "the dilator nerve of the cardia" and showed that contraction or relaxation could be obtained on stimulation of the vagus nerve. He suggested that the response was associated with the frequency and strength of the stimulus. Courtade and Guyon (44) invariably obtained a motor effect on the longitudinal fibres of the cardia and sometimes on the circular fibres: a relaxation of the circular fibres occurred, however, in some experiments. Langley (91) was unable to obtain any response of the fundic region until after injection of atropine, when he not infrequently obtained relaxation of the whole fundic end of the stomach. Relaxation of the cardia was also noted, especially if tonus was high. May (102) described a relaxation of the cardia followed by a slow augmentor effect, but at times a purely motor response resulted. Klee (79, 82), using x-rays found that the cardia remained closed: Koennecke (84) also obtained closure of the cardia, while Meltzer and Auer (117) agree with the findings of May and report a dilatation followed by a contraction. Carlson, Boyd and Pearcey (36) found that the vagus exerts both motor and inhibitor control over the cardia. These observers suggest that the type of response is dependent on tonus. Carlson and Litt (37) also reported that contraction of the cardia was obtained on stimulation of the vagus nerve. Caballero (28) disagrees with the previous results and states that the so-called "cardiac sphincter" is merely a valve against regurgitation, and that it is uninfluenced by nerve stimulation.

Body of the stomach. Van Braam Houckgeest (69) described in 1873 the effects of stimulating either nerve in the cervical region. The stomach, he says, contracts after a latent period of some four to five seconds: the movements being at first limited to the midstomach, but later peristaltic waves pass over to the pyloric region. Morat (118), and Doyon (49) note an increase of tonus on stimulation of the peripheral cut end of the nerve. Doyon pointed out that movements may continue for a long period after cessation of the stimulus, but only if one or both vagi are intact. He also obtained inhibition of movement and diminution of tone without any motor effect. The experiments of Morat (118) and Wertheimer (148) are confirmatory as they show that

the vagi may serve as the efferent path of inhibitory reflexes. Klee (79, 82) only observed motor effects, but found augmentation of peristalsis with a moderately strong stimulus and tonic contraction with a strong stimulus. Bechterew and Mislawski (13), Meltzer and Auer (117) recorded inhibition and associated the response with the strength of current. Kelling (78) working on rabbits and dogs observed that tonus fell whilst contractions were occurring, and stated that the vagus nerves exerted a considerable influence on the tension of the muscle fibres, but that vagal stimulation could not bring about a permanent increase of tonus.

Carlson and Luckhardt (38) working on the stomach of the turtle obtained on faradic stimulation a preliminary contraction followed by relaxation. Bercovitz and Roger (15, 133), and later Bercovitz (14), confirmed these observations. They state, however, that in the turtle no method of increasing gastric tonus is available by stimulation of the vagus: with a galvanic current they obtained either slight inhibition or a negative result.

The experiments of Cannon and Lieb (34) are of considerable interest, as their observations confirm the statements that inhibition of the stomach may take place on stimulation of the nerve. They noted that the receptive relaxation which accompanies deglutition is lost if both vagi are cut. Danielopolu (46) observed that compression of vagus in the neck of man caused inhibition followed by augmented movement.

More recently, McCrea, McSwiney and Stopford (109) showed that stimulation of the peripheral cut end of either vagus may either initiate movements, if the organ be in a state of "hypotonus," or, if movements are present, augment and sometimes accelerate them. If the muscles, on the other hand, be initially in a state of "hypertonus" movements, if present, are inhibited, and relaxation occurs. Augmented or initial contractions ensue later, but may be delayed until after the cessation of stimulation. These experiments are in agreement with the findings of Courtade and Guyon (44), and Carlson, Boyd and Pearcey (36) who also used the condition "tonus" to explain their results. Patterson (126) has more recently found a relation between the response and the condition of tonus. Similar results were obtained on stimulation of the central, as for the peripheral end of the vagus nerve. Laughton (94) has also shown that stimulation of the dorsal vagus nucleus usually causes contraction; relaxation was obtained, however, if tonus was high.

Veach (143), 1925, investigated the vagus effects on the lower end of the esophagus, cardia, and stomach of the cat, and the stomach and

lung of the turtle, in relation to Wedensky inhibition. Cannon (33), in preliminary experiments, had observed that low frequencies and intensities of stimulation of the vagus nerve in the cat had a pronounced motor effect on the cardiac sphincter, whereas higher frequencies and intensities were inhibitory. Veach found that stimulation of the peripheral end of either vagus with relatively low frequencies or intensities has motor effects on the lower end of the esophagus, the cardia and the body of the stomach of the cat. Stimulation with considerably higher frequencies or intensities has inhibitory effects on these structures, the inhibition being preceded as a rule by an initial contraction. Similar results were given by the stomach of the turtle. Veach and previous observers are agreed that the results are unaffected by removal of the suprarenals or section of the splanchnic nerves. From these results the explanation is advanced that the inhibitory effects are the results of a phenomenon allied to Wedensky inhibition.

McSwiney and Wadge (116) investigated this question and were unable to confirm the relationship suggested by Veach between the strength and frequency of the stimulus to the response. They found that in conditions of low tonus, stimulation of the vagus with low or high frequencies or intensities causes contraction of the stomach and an increase in tonus. In conditions of high tonus, stimulation of the vagus with low or high frequencies or intensities causes relaxation. Nolf (123) has also recorded inhibition with currents of low intensity, particularly in conditions of high tonus.

Veach, Schwartz and Weinstein (145) disagree, however, with the findings of McSwiney and Wadge. In a paper published in 1930 they figure tracings:

1. To show that a low frequency produces a steadily developing motor effect with relaxation on cessation of stimulation to a tonus level slightly lower than that preceding stimulation. With a higher frequency a brief contraction was followed by pronounced inhibition during stimulation, and on cessation of stimulation a pronounced after-contraction.

2. To show that an increase in frequency of stimulation is sufficient to change a motor effect into inhibition.

It may be pointed out that the motor effects illustrated are both of the same nature, a small steadily increasing motor effect. These observers suggest that as the term "Wedensky inhibition" is not especially descriptive, it might be well to replace it by the phrase "inhibition through fatigue." It is understood that this fatigue involves only a

slight conducting link over which the propagated disturbances pass to the affector, and this link is probably in or near the neural region of Langley.

While Veach, Schwartz and Weinstein have shown that a steadily developing motor effect and inhibition may be obtained by variation of the frequency of stimulation independent of tonus, the association between the condition of tonus and the response of the organ was not investigated. Brown and Garry (23) have, however, in this laboratory recently re-investigated the condition. As Garry (61) had shown that sodium amytal paralyses the inhibitory response of the heart to stimulation of the vagus nerve, it was thought that that use of this drug might afford useful information. Brown and Garry found, using the decapitate cat, that after injection of sodium amytal the inhibitory response to vagus stimulation was lost, a few break shocks or faradic stimulation causing contraction of the stomach independent of the condition of tonus. Further, on stimulation of the vagus fibres below the heart before injection of amytal, they not infrequently obtained relaxation with low frequencies, while on increasing the frequency of stimulation, contraction of the stomach was recorded. It would appear, therefore, from these and other experiments quoted later, unlikely that the inhibitory effects are related to Wedensky inhibition or inhibition through fatigue.

Pyloric antrum and sphincter. Many isolated observations have been made on the pyloric sphincter, but few observers appear to have considered the sphincter in conjunction with the antrum and the remainder of the stomach. Many of the isolated findings are in direct contradiction to each other.

Van Izeren (72) and Oser (125) obtained contraction and closure of the pylorus,while Koennecke (84) observed opening. Rossbach (134) and Langley (91) are in agreement, and noted sometimes contraction, sometimes relaxation. May (102) described relaxation followed by rhythmic contraction and finally closure. Klee (79, 82) observed increased initial emptying of the stomach, and Nakanishi (120) obtained relaxation, particularly if tonus was high. Courtade and Guyon (44) noted relaxation of the circular muscle fibres followed by rhythmic contraction; the sphincter usually functioned with the pars pylorica. Thomas and Wheelon (142) found that an abrupt rise of tonus occurred with increase in the rate and force of the normal movements. They observed that the sphincter functioned with the pyloric antrum. Carlson and Litt (37) observed contraction of the pylorus with low or moderate tonus and occasionally relaxation in the hypertonic or spastic stomach.

Cole (42), Luckhardt, Phillips and Carlson (98), Wheelon and Thomas (150) have, amongst others, observed the relationship between the movements of the antrum and the pyloric sphincter. Pyrah (128) working in this laboratory on the regulation of the sphincter confirms the general observations of Wheelon and Thomas. McCrea and McSwiney (106) separated the antrum from the remainder of the stomach and studied the response of these regions to stimulation of the vagus nerve. They were able to show a complete dissociation in the response of the fundus and antrum. In the inactive antrum, movements were initiated, while in the active contracting antrum, contractions were usually inhibited.

Consideration of the results obtained by different observers shows that stimulation of either vagus by a faradic current results in *a*, contraction or contractions, or *b*, inhibition or relaxation. Some observers find a greater effect is obtained with the left than the right vagus. The majority of workers find that tonus is increased, though some have noted a decrease. Several observers have related the type of response to the condition of tonus, while others find that the response depends on the strength and frequency of stimulation.

Vaso-constrictor effects evidenced by blanching of the stomach have been observed, but it is doubtful whether this can be considered a pure vasomotor effect (Latarjet, Cluzet and Wertheimer, 93), as the blanching is usually associated with powerful contractions. Decerebration, decapitation, pithing of the cord, section of the splanchnics, removal of the suprarenals do not materially affect the results, but the anesthetic, and method of experimentation are important. Deep ether anesthesia or excessive handling of the organ may produce marked dilatation and shock of the organ. Again, some anesthetics, e.g., sodium amytal, may inhibit contraction or relaxation. Blood pressure, vasomotor changes, asphyxia, and diaphragmatic movements have been excluded as a source of error.

From the evidence it would appear that there is an association between the type of response and the condition of tonus. On the other hand, it is clear that contraction or relaxation may be obtained with different strengths and frequencies of current without postulating a mechanism of the nature of Wedensky inhibition.

Effect of stimulation of the sympathetic. The splanchnic nerves are almost universally stated to be the inhibitor nerves of the stomach, but some observers have recorded augmentor effects. Although some observers have concluded that these nerves convey impulses which may cause inhibitor or augmentor effects, the statements

that they are pure inhibitor nerves have been allowed to pass almost unchallenged. The conception, as put forward by Gaskell, has been wholeheartedly accepted, and the evidence has not been carefully sifted.

Van Braam Houckgeest (69), 1873, obtained inhibition with cessation of movement immediately on stimulation, but Bechterew and Mislawski (13) recorded that stimulation did not always result in inhibition. Oser (125) found a weak contraction occurred, which was followed by a relaxation of long duration, during which existing movements were inhibited. An increase in peristalsis was observed as an after-effect which could be arrested by further stimulation. Morat (118) stated that either inhibition or augmentation could occur. Doyon (49) also observed inhibitor and augmentor responses. He found that if both vagi were divided, the stomach then being quiescent, stimulation results in a single contraction, while if the stomach is active and contracting, then stimulation causes inhibition. Batelli's (9) results are confirmatory, as he also obtained contraction of the organ. Courtade and Guyon (44) found that splanchnic stimulation results in relaxation and inhibition of movements of all parts of the stomach. Elliott (56) also obtained pure inhibitory effects with the exception of the pyloric sphincter. Klee (80), using x-rays, also invariably found inhibition. Carlson, Boyd and Pearcey (36) observed, on the other hand, in their studies of the cardia, both motor and inhibitor effects in the cat; in the dog, motor; and in the rabbit, inhibitor.

Thomas and Wheelon (142) have also recorded motor and inhibitor responses. They observed that an inhibitor effect is often associated with a rise of blood pressure, and an augmentor with a slight rise: reversal effects were also obtained. Nolf (122), in 1925, confirmed Doyon's earlier observations. Veach (144), 1925, obtained contraction and relaxation of the stomach, and attributes the results to the degree of vaso-constriction present. If vaso-constriction is slight or non-existent, contraction often occurs: if great, relaxation results. McCrea and McSwiney (107) found that stimulation of the peripheral cut end of either splanchnic nerve will, if the organ be in a state of "hypotonus," bring about a rise in the entogastric pressure. Should the viscus be in a condition of "hypertonus," a fall of entogastric pressure takes place, and existing movements cease. Brown, McSwiney and Wadge (26) working with spinal and decerebrate cats and dogs, the stomach being divided at the incisura, found the effect on the stomach was associated with the type of stimulation employed. Stimulation at a frequency of 1 per second caused contraction of the body of the stomach, while

stimulation with a tetanizing current brought about relaxation. The movements of the cat's antrum were inhibited by all forms of stimulation. The antrum of the dog, although usually inhibited, occasionally showed an augmentor response to sympathetic stimulation. The motor responses were usually inhibited by ergotoxine. Adrenaline did not, however, reproduce the effects of sympathetic stimulation. Recently, Brown, McSwiney and Wadge (27) have investigated the effects of injection of sodium luminal. They find, using the decapitate preparations, that after injection of the drug, sympathetic stimulation of any strength or frequency of current causes contraction. The inhibitor response, which previous to injection of the drug was predominant, is converted into an augmentor response. Ergotoxine abolishes the contraction, and further stimulation of the nerve may cause either no effect or slight inhibition.

Pyloric region. Contejean (43), 1893, and Elliott (56) have described movements of the pyloric region on splanchnic stimulation. Oser (125), on the other hand, observed relaxation of the sphincter. Nakanishi (120) found that contraction occurred, but was followed by relaxation. Carlson and Litt (37) find that the atonic or moderately tonic sphincter is contracted, while they sometimes observed the hypertonic to be relaxed. Brown, McSwiney and Wadge (26) obtained inhibition of movements of the separated pyloric antrum in the cat. In the dog, inhibition was most frequently observed.

It is clear from perusal of the literature that the sympathetic cannot be considered as a pure inhibitor nerve of the stomach. Augmentor and inhibitor responses have been recorded by most investigators. A correlation between the existing condition of tonus and the response of the organ has been made by many observers: Doyon (49), Nolf (122), Carlson, Boyd and Pearcey (36), McCrea and McSwiney (107). Thomas and Wheelon (142) and Veach (144) have correlated their findings with blood pressure, while Brown, McSwiney and Wadge (26) associate the response with the strength and frequency of the stimulus.

NERVE MUSCLE PREPARATION. Recently (129) it has been possible to prepare isolated preparations of mammalian smooth muscle supplied by the vagus and sympathetic nerves (113). The response of the smooth muscle of the stomach on stimulation of the vagus nerve with single and repeated stimuli has been recorded. The average latent period equals 0.77 second, contraction 2.2 seconds, and relaxation 6.2 seconds.

Peripheral summation of stimuli has been demonstrated, and summation of contraction was recorded with two or more stimuli. The time

intervals at which summation was obtained agree with the observations and hypothesis of Bremer (20). The contraction evoked by stimulation of the nerve was converted into relaxation by addition of atropine, which confirms Langley's (91) original observation. Peripheral summation is suggested to be due to synaptic junctions in Auerbach's plexus, which confirms the observations of Gaskell, Hill and others.

McSwiney and Robson (114) have shown that stimulation of the peri-arterial (sympathetic) nerves with frequencies of one to twelve break induction shocks per second caused contraction of the muscle: with twenty to fifty shocks per second, relaxation was obtained. Reversal effects were also recorded by varying either the strength of current or the duration of faradic stimulation. The latent periods for contraction varied between 9.2 seconds and 1.4 seconds, the average being 4.8 seconds: for relaxation, the times varied between 4.2 and 2.3 seconds, the average being 3.3 seconds. These results confirm the previous observations of Brown, McSwiney and Wadge (26), and show that contraction and relaxation are not directly related to vasomotor changes nor dependent on the existing state of tonus.

McSwiney and Robson have recently investigated the effect of vagus stimulation on the response of the muscle to stimulation of the peri-arterial or sympathetic fibres (115). It has been found that stimulation of the sympathetic at a frequency of twelve break shocks per second causes contraction. Stimulation of the sympathetic at the same frequency during the contraction produced by a tetanization of the vagus is followed by relaxation. Reversal of the sympathetic response also takes place if the nerves are stimulated during vagus stimulation, but after the muscle has returned to its resting length through apparent fatigue. Again, certain types of sympathetic stimulation may in ordinary conditions have no effect on the muscle. The same stimulus applied during vagal stimulation may produce relaxation.

Evidence may also be obtained of the inhibitory action of the sympathetic on the vagus. Stimulation of the sympathetic causes contraction. If during the sympathetic stimulation the vagus is also stimulated, a further contraction ensues. Sympathetic stimulation is then discontinued, and the vagal contraction immediately increases in magnitude. It has been found that the longer the duration and the higher the frequency of sympathetic stimulation, the greater the inhibitory effect on the vagus.

Of interest are the recent observations made by Dickinson, McSwiney and Robson (47) on isolated nerve muscle preparations of the tortoise.

On stimulation of the vagus nerve with a faradic current, the muscle usually contracts, but relaxation has also been recorded. The conditions that determine the type of response have not yet been defined. Further, the preparations show a considerable variability in the time relations of contraction and in length changes. The latent period at 17 to 18°C. is usually of the order of 20 seconds, but latent periods varying between 5 and 38 seconds have been obtained. The time relations of contraction are markedly affected by temperature. A latent period of 20 seconds at 17 to 18°C. is of the order of 60 seconds at 10°C. and 10 seconds at 25°C. To obtain a response from the muscle an interval of 15 minutes must usually be allowed to elapse. Addition of acetyl choline has been found to cause a marked contraction.

Qualitative observations on the relations between vagus and sympathetic stimulation have previously been made in the intact animal. Batelli (9) and Oser (125) found that simultaneous stimulation of both vagi and splanchnic nerves reduced the effects of exciting the vagi. Oser observed that the vagus action first appeared, but that, if in place of simultaneous stimulation, the splanchnics were stimulated first so that inhibition was predominant, then a vagus effect was difficult to obtain. McCrea and McSwiney (107) also noted that vagal responses are much diminished after splanchnic stimulaton.

THE ACTION OF DRUGS. Smith (137) has made a detailed study on the action of drugs on surviving strips of the stomachs of guinea pigs, cats, rabbits, dogs and man. Pilocarpine caused contraction of all parts of the stomach in all animals. This contraction was inhibited by atropine. Adrenaline caused relaxation of all regions in the cat except the sphincter. In the dog, adrenaline caused relaxation of the preantrum, no response from the antrum, and contraction of the body and fundus.

Hecht (64) observed that the action of atropine on the complete surviving stomach of the cat is one of excitation or inhibition, the type of reaction depending upon the absence or presence of peristaltogogues in the wall of the gut, confirming the work of Le Heux (65) on the intestine.

Tezner and Turolt (140) recorded the inhibitory effects of atropine and adrenaline on strips of the human stomach. Kuroda (89) has recorded the behavior of longitudinal muscle strips from the fundus of the dog's stomach. Acetyl choline caused contraction, but was without action after atropine. In the majority of instances, adrenaline caused contraction. Brown and McSwiney (24) investigated the action of

pilocarpine, adrenaline and atropine on surviving strips of the gastric musculature of the cat and dog. Pilocarpine imitates the motor action of the vagus nerve, while adrenaline imitates the inhibitory effects of the splanchnic nerve in all regions of the stomach except the cardia, in which it causes contraction. Atropine antagonizes the action of pilocarpine, and produces effects similar to those observed after adrenaline.

The experiments made on the reaction of strips of the rabbit's stomach to adrenaline are of interest (25, 112), as they explain the various effects recorded by previous observers. It was shown that addition of adrenaline to strips from the fundus and body of the stomach may have an excitatory or inhibitory effect. The usual response of the muscle is one of contraction; if, however, the muscle fibres are shortened by pilocarpine, choline, histamine, pituitrin or barium chloride, an inhibitory response is obtained with adrenaline. An inhibitor response may be changed into an augmentor response by certain agencies, as atropine, causing a lengthening of the muscle fibres. Strips of the dog's fundus may be contracted by adrenaline if the preparations are left in cold Ringer-Locke solution for 2 to 3 hours. The results obtained with adrenaline on the rabbit's fundus resemble closely the effect of stimulation of the vagus and sympathetic nerve in the isolated nerve muscle preparation previously described.

The description of the action of drugs on the organ "*in situ*" is important, as the results obtained may be compared with the effects of stimulation of the vagus and sympathetic nerves.

Doyon (50) and Batelli (9) observed the stimulating action of pilocarpine, but Batelli stated that the inhibitory fibres of the vagus are excited. McCrea and Macdonald (105) found that pilocarpine and acetyl choline can be both augmentor and depressor to the stomach according to the condition of tonus.

Elliott (56) and Cannon (32) both recorded the inhibitory action of adrenaline. Carlson, Boyd and Pearcey (36) demonstrated, however, that the effects obtained with the drug on the cardia were similar to those previously described on stimulation of the nerve fibres. Adrenaline had a motor action when the stomach was hypotonic and an inhibitory action when hypertonic. Thomas (141) obtained similar results working on the pyloric region of the dog, though Smith (137) states that the sympathetic nerve supply to the pylorus is excitor. McCrea and Macdonald (105) have more recently shown that adrenaline may produce both inhibitor and augmentor effects on the stomach of the dog and cat, but its action is chiefly inhibitor. They state that the action

of the drug cannot be said to duplicate the effects of faradic stimulation of the splanchnic nerves. An augmentor effect was only occasionally obtained in the cat and then with large doses. An augmentor effect was, however, more easily elicited in the dog. These results, it is true, do not agree with the hypothesis that the effects of sympathetic stimulation are dependent on the condition of tonus, but more recently, as has been previously described, Brown, McSwiney and Wadge (26) have demonstrated that inhibitor and motor effects can be obtained by varying the strength and frequency of the current.

Langley (91) recorded reversal of the action of the vagus nerves after injection of atropine. Carlson, Boyd and Pearcey (36), Batelli (9), McCrea and Macdonald (105) agree that atropine paralyses the gastric musculature, eliminating both vagus and splanchnic control.

For the literature dealing with choline as a gastro-intestinal hormone, reference should be made to a review by Magnus (100) published in 1925.

Discussion. It is possible from the experimental findings to divide the stomach functionally into two parts:

a. The upper body and fundus which show a permanent shortening with little rhythmic augmentation.

b. The lower body and antrum which react with great augmentation of movement, and with little or no change in the permanent length of the muscle fibre.

The movements of the stomach, as observed in man, dog, and rabbit, are usually of the two-phase type, while the one-phase type as described by Cannon (29) is normally observed in the stomach of the cat. Either form, however, may on occasion be found in the stomach of man, cat and rabbit. Alvarez (4) points out that the point at which the waves change into systole varies in different animals and from time to time in the same animals.

The sympathetic and parasympathetic nerves regulate two different types of response of the stomach:

1. Pure tonus changes, as evidenced by contraction or relaxation of the muscle fibres.

2. Augmentation or inhibition of rhythmic movements.

It would appear, however, that the immediate effects of vagotomy, splanchnotomy, and denervation of the stomach are similar, namely, retardation of function. After a period the peripheral intrinsic nervous mechanism assumes control and only one permanent symptom, "decrease in initial emptying time" is observed. While the vagus and sympathetic nerves are essential for the normal regulation of the organ

in association with other functions of the body, the denervated stomach may function adequately. On the introduction of food, movements are initiated, and the digested material is passed on to the next region of the digestive tract.

Evans (57) defines the tonus of plain muscle as the resistance of its substance to extension. He further points out that if contraction be regarded as the consequence of certain chemical and physical changes in the cell, and relaxation as their reversal to a former state, there should be no difficulty in considering a state of tonus either as a continued contraction or as an inhibition or partly inhibited relaxation. From such a new equilibrium a state of further contraction or further relaxation could be reached. Such an hypothesis assumes that the "all or none" law does not hold for plain muscle fibres. On the other hand, Ritchie (132) has made the interesting suggestion that the phenomena as they stand could be accounted for without assuming any special "catch" mechanism for the tonic contraction, if it were assumed 1, that passive stretch is a stimulus; 2, that there is a long refractory period after contraction; 3, that pure tone without rhythmic movement is due to a completely synchronous contraction; rhythmic movement without tone to completely synchronized contraction. In pure tonic contraction it is assumed that a small number of fibres distributed through the tissues are active at one time. Their relaxation, when fatigue has lowered their excitability, will stimulate the most excitable by passive stretch, the most excitable fibres being those that have had the longest time to recover since previous activity. So the activity will go on in rotation keeping the whole tissue of constant length, as long as the excitability and rate of recovery from the refractory state remain constant.

Evans has confirmed the findings of previous observers that the maintenance of the tonic state is not associated with any increased expenditure of energy. The phenomena postulated by Ritchie do not suggest an increased oxygen consumption; indeed Bayliss (10) calculates that the observed oxygen consumptions of mammalian smooth muscle do not necessitate the assumption of a mechanism for tone other than tetanus, if the time relations of the contractile process are considered.

Rhythmic contractions, which are a common phenomenon of plain muscle, are well marked in the pyloric region and body of the stomach. Stimulation of the vagus and sympathetic nerves may either increase or decrease these contractions. The myogenic and neurogenic theories have each been advanced to account for the movements, but the most

satisfactory evidence appears to be in favour of the myogenic nature of the spontaneous contraction, as Verzar (146) has shown that the muscle cells of the chick's amnion, devoid of nerve fibres, exhibit rhythmic contractions. For evidence on this aspect, reference should be made to Evans (57) and Hill (66), who have dealt fully with the subject.

It is doubtful whether an explanation based wholly on stretch reflexes, as put forward by Ritchie (132), can account for the regulation of the tonus mechanism of the stomach. The evidence available of the effects of different foodstuffs on the motility of the stomach suggests that chemical substances may also play a part in augmenting or inhibiting the contractions. Such an hypothesis must, however, be put forward very tentatively, in view of the complex nature of the problem and the few observations available.

The hypotheses advanced to account for augmentor or inhibitor responses of plain muscle of the stomach to stimulation of the vagus and sympathetic nerves are at present incomplete, and it is obvious that the vagus cannot be considered as the motor, nor the sympathetic as the inhibitor nerve, since stimulation of either nerve may cause contraction or relaxation of the organ.

From the histological evidence of the enteric plexuses and nerve endings, it would appear that the vagus nerves end in arborizations around cells of Auerbach's plexus, while the postganglionic fibres of the sympathetic end in association with the muscle fibres. Summation of stimuli demonstrated by McSwiney and Robson (113) in vagus nerve smooth muscle preparations support the histological evidence of synaptic junctions in the enteric plexuses.

The different results obtained on stimulation of the vagus are explained by Veach (143) on the basis that tonus is dependent upon the passage of propagated disturbances over a peripheral part of the neuromuscular mechanism, and the degree of tonus upon the frequency of these propagated disturbances. Thus, stimulation of the vagus when tonus is high will increase the frequency of the propagated disturbances to an inhibitory value. On the other hand, when the tonus is low, the frequency of the existing propagated disturbances in the peripheral mechanism is also low: stimulation of the vagus will then only raise the frequency of the propagated disturbances to an excitatory value.

In view of the close analogy of the reactions to Wedensky inhibition, they are accounted for on the basis of a reduction of propagated disturbances to a subthreshold magnitude in a conducting mechanism of the smooth muscle fibres, as a result of each travelling in the relatively re-

fractory phase produced by its predecessor. It is suggested that escape from inhibition may be accounted for, in part at least, on the basis of increased synaptic decrement, possibly associated with a decrease in the magnitude of propagated disturbances. Veach (144) holds with other observers that the effects of sympathetic stimulation are dependent on the degree of vaso-constriction of the blood as evidenced by the pressure.

The explanation based on "Wedensky inhibition" or "inhibition through fatigue" is not borne out by the experimental evidence. Details are given in this review of investigations on the intact animal, isolated nerve muscle preparations and the effect of the addition of drugs, which do not support the theory. Further, the hypothesis has not been established for the sympathetic nerves, and it is extremely unlikely that two widely differing mechanisms would exist for the different nerve fibres.

The observations made on the relation of tonus to the type of response do not afford an explanation. These experiments simply demonstrate that with certain conditions of tonus, contraction or relaxation, or again inhibition or augmentation, is the more likely response.

Consideration of the evidence which is being accumulated from stimulation of the sympathetic and parasympathetic nerves supplying various organs demonstrates that a definite foundation is being laid for the explanation of the results on the basis of liberation of chemical substances at the nerve endings. Loewi and his co-workers have demonstrated that the inhibition of the frog's heart by the vagus is due to liberation of a substance which has all the properties of an unstable ester of choline. Brinkman and Van Dam (21) showed that the substance liberated by the heart vagus not only inhibited the activity of the heart muscle, but stimulated that of the stomach. Brinkman and Van der Velde (22) have also demonstrated that arterial blood withdrawn from a rabbit after stimulation of the vagus nerves causes augmentation of the stomach, when injected into the ear vein of a second animal. Dale and Gaddum (45) have recently put forward evidence supporting the view that the vaso-dilator effects of the parasympathetic nerves and of sensory fibres stimulated antidromically, and the contractures of denervated muscles accompanying these actions, are due to the liberation of acetyl choline. These observers point out that the suggestion of humoral transmission for certain effects can logically be applied to all their effects, which are chiefly obtained with plain muscle. The responses of denervated voluntary muscle and plain muscle to nerve

stimulation show features which recall the phenomena of "recruitment" and "after discharge" as described in the spinal animal.

Direct evidence of the peripheral liberation of an inhibitory substance has been afforded by the experiments described by Finkleman (59). He showed that if the movements of a piece of intestine are inhibited by stimulating the periarterial nerves, a substance appears in the fluid which has the power of inhibiting a second piece of gut. While, at present, we possess no other direct evidence that chemical substances are produced at the nerve endings, it is difficult to see how the results obtained from nerve stimulation *in vivo* and *in vitro* can be explained on any other basis. The delay in onset, the rate of development of contraction, the reversal of the response with varying spacing of shocks, the effect of sympathetic stimulation on vagus contraction afford much evidence in favour of the hypothesis.

From the experimental evidence it is possible to postulate the presence of motor and inhibitor fibres in the vagus and sympathetic nerves, and this hypothesis receives some support from the experiments which have been made on the vasomotor nerves to demonstrate the existence of vasodilator and vasoconstrictor fibres (130). Further, it might be suggested that the effects observed are due to the nerve fibres regulating two types of muscle cells, one type responsible for contraction, the other for relaxation; but when we carefully examine the experimental results, it becomes apparent that the evidence for the presence of motor and inhibitor fibres is still insufficient to justify any conclusion, and no observations have been made, as yet, on this type of plain muscle which demonstrate different functional forms of muscle cells.

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ADDENDUM

Kuré and his co-workers (8, 9, 10, 11, 12, 13) describe in their publications numerous small myelinated efferent fibres in the dorsal spinal roots constituting what they term "the spinal parasympathetic." These fibres remain undegenerated in the central end of a divided dorsal root. Recently, Kuré, Ichiko and Ishikawa (9) have stated that "the spinal parasympathetic fibres" supply the

stomach and intestine through the greater splanchnics. Both splanchnics have an excitatory action on the stomach when the inhibitory fibres are interrupted by the application of nicotine to the coeliac ganglion.

Cajal (2), Lenhossék (14), and van Gehuchten (5) have observed, by the Golgi method, nerve cells situated within the spinal cord in the chick, the axis cylinders of which pass out in the dorsal roots. Cajal (3) and Kölliker (7), however, state that these fibres have not been found in other animals.

Sherrington (17, 18) found, thirty-three days after excision of the fourth to the ninth post-thoracic dorsal root ganglia and again forty-two days after excision of the fourth to the eighth post-thoracic dorsal root ganglia, numerous (several thousands) finely myelinated fibres 1μ to 3.5μ in diameter present in the proximal stump of the dorsal root. In animals, however, in which the fourth and fifth post-thoracic nerves were severed close to the ganglion, the proximal stumps contained no undegenerated medullated fibres less than 12μ in diameter. A few large fibres about 20μ in diameter were not sufficiently altered to allow an opinion to be formed.

The small myelinated fibres resembled regenerating fibres for the following reasons: (1) the excessive thinness of the myelin sheath, (2) the conspicuous character of the nodes of Ranvier, (3) the frequency of occurrence in couplets and triplets, (4) the somewhat tortuous course, (5) the appearance of being embedded in or on paler, embryonic, somewhat flattened, "protoplasmic" fibres.

Some early investigators, Joseph (6), Singer and Münzer (19), claim to have observed degenerated fibres in the peripheral stump, but Ranson (16) and Nevin (15) fail to confirm Kuré's histological findings and confirm the results obtained by Sherrington.

The method used by Ken Kuré consisted in counting myelin sheaths in cross section, which does not overcome the objection that small resistant segments of a degenerating fibre will give a circular cross section for long periods. On the other hand, Sherrington, to demonstrate the fibres, teased short segments (0.5 to 1.0 mm. long) cut from the dorsal root after fixation in osmic acid and maceration in 33 per cent alcohol—a method by which one sound fibre can be recognized among hundreds of degenerating fibres.

Denny Brown (1) using cats has confirmed the observations of Sherrington and others. He is satisfied that examination of the proximal stump of the dorsal root fourteen, seventeen and twenty-one days after operation revealed no completely sound fibres. A very large number of finely myelinated fibres were, however, observed seventy-five days after excision.

It therefore seems certain that the finely medullated fibres in the proximal stump of the dorsal root enter the root in some manner between the third and fourth week and increase steadily in numbers and, to some extent, in size after that period. Tower (20) in recent studies finds that the fibres in the cat enter the cut extremity of the root from the scar where the ganglion has been excised, and penetrate the root up to the level of the cord. The fibres end in wandering loops or knobs without apparent functional connection. The fibres were apparently ventral root fibres which had sprouted up the degenerating stump of the dorsal root.

Cajal (4) has shown that degenerating fibres have a powerful attraction for regenerating sprouts, and, further, that the bands of Büngner can attract sprouts

from apparently undamaged nerves. In the present instance, the degenerating dorsal root apparently provides the stimulus, and the results are similar whether the ganglion has been excised or the root simply sectioned near the ganglion.

Kuré's findings, in view of the studies of Nevin, Sherrington, Ranson and others, must be received with caution. It may be pointed out that the distribution of the nerve fibres does not correspond with the observations of other authors. The technique and experimental methods employed by Kuré and his co-workers are certainly open to criticism, as no tracings or records were made. The nerve fibres were traced and the function of the roots investigated by making direct observations on the movements of the stomach and intestine.

I am indebted to Dr. Denny Brown for his assistance in preparing the addendum.

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THE PRESENT STATUS OF THE CHEMISTRY OF SKELETAL MUSCULAR CONTRACTION

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At present the investigation of the chemical changes which accompany skeletal muscular activity is being actively prosecuted and the subject is one about which nothing approaching a final statement can be made. The literature up to the beginning of 1930 has been very fully and critically discussed by Meyerhof (49) and as, up to this time, attention had been mainly directed to the carbohydrate changes associated with the production and removal of lactic acid, it is perhaps advisable in this article to consider more fully the recent work in which mechanisms other than the glycolytic one play the predominant rôle. As changes in these pre-lactic acid processes are among the earliest ones to appear, even when the carbohydrate metabolism is proceeding along normal lines, they may quite reasonably be dealt with at the outset. Up to the present the four constituents which have been recognised as undergoing changes in vertebrate skeletal muscle under different conditions are 1, creatine-phosphoric acid (or phosphagen); 2, adenosine triphosphoric (or adenyl-pyrophosphoric) acid; 3, glycogen, and 4, the hexose monophosphate "lactacidogen." Under normal conditions all four are concerned in the chemical changes which accompany muscular activity. It is possible to limit the changes mainly to the first two and hence it is of importance to consider not only the normal carbohydrate cycle, but also the transformations which take place in active muscle when the energy changes are associated with chemical processes other than those concerned with lactic acid production. Even when contractions occur without lactic acid production, the carbohydrate does undergo changes in so far as the phosphoric acid set free from the phosphagen and the adenyl-pyrophosphoric acid is in part used up for the esterification of the hexoses formed from the enzymic hydrolysis of the glycogen. Contractions without the appearance of lactic acid, or with a very small production, have been studied 1, under conditions when the glycogen store has not been diminished and in which glycogenolysis and hexose esterifi-

cation take place without the final transformation to lactic acid, and 2, in cases where the glycogen store has been practically exhausted. It is to the former that attention has recently been mainly directed. Before considering the "alactacid" contractions it is necessary to refer briefly to the components in muscle which are concerned in the chemical processes associated with normal muscular activity.

1. CREATINE-PHOSPHORIC ACID OR PHOSPHAGEN. In Eggleton's paper in these REVIEWS (10), the investigations dealing with the general characters and the distribution of the phosphorus compounds in muscle are dealt with up to 1928, so that it is not necessary to do more than refer briefly to the earlier work on phosphagen. In a preliminary communication in 1927, and much more fully in a later one in 1929, Fiske and Subbarow (22, 23) brought forward definite evidence that the labile phosphate compound in muscle (phosphagen of the Eggletons) contained phosphoric acid and creatine in equimolecular amounts, and described methods for its separation and estimation. In order to arrive at a knowledge of the relationships of the free phosphate and free creatine to this compound in muscle, it is advisable to consider the equilibrium concentrations in which they exist in muscle under different conditions. The total free and labile phosphate in muscle is approximately 0.029–0.036 molar, and the total creatine is approximately of the same molar concentration or rather less, 0.025–0.030 molar, so that practically all the orthophosphate, which can be directly determined in muscle, might be loosely bound to the creatine present. The concentration of free diffusible phosphate in muscle under different conditions has been determined by Stella (69) who found that, in the case of dead amphibian muscle, phosphate equilibrium was established when the external saline solution had a concentration of approximately 110 mgm. P per cent (0.036 molar). It appears therefore that all the orthophosphate, which can be chemically determined in dead muscle, is readily diffusible. In the living resting muscle, on the other hand, the equilibrium concentration is 8 mgm. per 100 cc. (0.0025 molar) and in fatigued muscle 18 mgm. per 100 cc. (0.006 molar). Eggleton (11) has recently calculated the creatine equilibrium concentrations and finds them to be for resting muscle 80 mgm. per 100 cc. calculated for the dispersion medium of muscle, or 65 mgm. per 100 grams muscle substance (0.0061 and 0.005 molar respectively). In fatigued muscle the similar equilibrium concentrations are 0.015 and 0.023 molar. It is evident that the equilibrium concentrations of the two components of the phosphagen are not the same in the living muscle whether it be resting or fatigued.

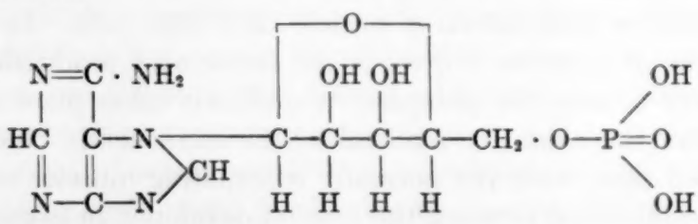
The molar concentration of diffusible creatine is higher than that of the diffusible phosphate. The free phosphate concentration is evidently kept down by some form of linkage other than creatine-phosphate which renders the phosphate indiffusible.

Phosphagen is extremely unstable in aqueous acid solutions even at low temperatures. Extracts of muscle and the expressed muscle juice contain an enzyme which rapidly breaks down the body at reactions between pH 6.4 and 7. At more acid reactions enzymic hydrolysis is delayed and still more so at alkaline reactions. At pH 9–10 a partial synthesis of phosphagen takes place and the synthetic effect is increased on the addition of glycogen, creatine or phosphate. Electro-titration curves clearly show that the creatine-phosphoric acid is a stronger acid in the bound form than in the mixture containing the dissociated components (fig. 1, p. 535). There are two acid dissociation constants, pK_1 2.7 and pK_2 4.5–4.58 (50), (22), (23). The curves show clearly the basic equivalents set free at different reactions. At the normal reaction of muscle the splitting of phosphagen can disturb acid-base equilibrium only to a slight extent, but at more acid reactions the setting free of basic valencies becomes more evident. The heat produced by enzymic or acid hydrolysis of purified phosphagen was calculated by Meyerhof to be 11,000 to 12,500 cal. per mol, the former in neutral and the latter in acid solution. The positive and negative heat values of enzymic phosphagen splitting and synthesis respectively in the natural protein holding extracts of muscle do not however correspond to those derived for pure phosphagen, owing probably to a difference in the form of combination in the protein medium.

CHANGES IN PHOSPHAGEN DURING MUSCULAR ACTIVITY. As the compound is such a labile one, the slight unavoidable excitation of muscle during its removal, and still more during its acid extraction, gives rise to a small amount of decomposition. This however becomes much more marked on stimulation of muscle (11), (12), (13). In the earlier contractions of a series it exceeds the lactic acid production, and in the recovery process the phosphagen synthesis takes place much more rapidly than the oxidative removal of the lactic acid. Nachmansohn (58) showed that, with the normally contracting muscle, no constant ratio was established between the tension developed in isometric single contractions or tetani and the amount of phosphoric acid set free. The rapidity of phosphagen splitting in the early stages of a contraction and the rapid partial synthesis of the body under anaerobic conditions are features which distinguish it from lactic acid production and removal.

The aerobic synthesis of phosphagen after hydrolysis is very rapid and unlike the anaerobic is complete. For these reasons Nachmansohn has endeavoured to associate the phosphagen metabolism with the velocity of the excitation process, and has tried to show a connection between the amounts and rate of breakdown of the body and the chronaxie values determined by the Lapicque method (59). Thus smooth muscle containing practically no phosphagen has a high chronaxie value while the more rapidly contracting pale muscle (low chronaxie) has a higher phosphagen content than the less excitable and more slowly contracting red muscle (higher chronaxie) Accepting Lapicque's views regarding the mode of action of curari, namely, a disturbance in the isochronism of nerve and muscle with a lengthening of the chronaxie of the latter alone, he suggests that the cause of this may be the check in phosphagen breakdown in excited curarised as compared to similarly stimulated normal muscle. This difference in the behavior of normal and curarised muscle on direct stimulation is most marked after tetanic stimulation. The significance of the phosphagen breakdown in the contraction phase will be subsequently discussed under "alactacid" contractions.

2. ADENYL-PYROPHOSPHORIC ACID. In 1847 Liebig (37) obtained from muscle a substance which he named inosinic acid and at a later period Haiser (29) recognised the existence of phosphoric acid in the molecule. Levene and Jacobs (33), (34), (35) finally established its structure as that of a mononucleotide with one molecule of hypoxanthine and one of phosphoric acid attached to d-ribose. In 1914 Embden and Laquer (14) detected the presence of a nitrogenous constituent in the hexose-diphosphate which they obtained from muscle under the action of fluoride, and the nitrogenous body was found to contain adenine. In 1927 Embden and Zimmermann (15) succeeded in separating the body in pure form and definitely established its structure as adenosine-phosphoric acid.



For the first time the relationship between the inosinic acid of Liebig and the adenylic acid of fresh muscle was definitely shown, the former being derived from the latter by enzymic deamination. Subsequently

the results of a series of investigations from the Frankfurt school (16) appeared, dealing with the formation of ammonia in muscular activity and under other conditions, confirming the view that the ammonia forming substance in muscle was adenylic acid. Embden found that the extra ammonia set free when adenylic acid was added to minced muscle corresponded to the amount derivable from the amino group of the adenine, the maximal yield of ammonia nitrogen amounting to one-fifth of the total nitrogen of the nucleotide. Schmidt (67) found two deaminating ferments in rabbit's muscle, one acting on adenosine, and the other on adenylic acid, while the free purine base was resistant. Yeast adenylic acid was not attacked by the muscle ferment. Parnas and Mozolowski (63), and later Parnas (64), carefully investigated the relationship between the ammonia formation and the changes in the nucleotide. They also recognised that the source of the ammonia was the adenylic acid, and that the amount of the adenine which disappeared in deamination corresponded closely to the hypoxanthine of the inosinic acid which took its place. This held good for the formation of ammonia under traumatic conditions and as a result of anaerobic fatigue. Parnas also investigated the relationship between ammonia formation and the deamination of adenylic acid in aerobic muscular activity. Here the deamination of the nucleotide lagged behind the ammonia production and, when the stimulation period was broken up by intermediate periods for oxidative recovery, the re-amination of the inosinic acid might be almost complete. He regarded however the ammonia production as an irreversible process, so that the ammonia set free could not be made use of in the muscle for re-amination, and considered that the aerobic conversion of inosinic to adenylic acid was probably brought about by the oxidative deamination of amino-acids. The changes might therefore be shown in the following form (65).

1. Contraction.
   Adenylic acid → Inosinic acid + NH_3
2. Recovery.
   Inosinic acid + O_2 + Amino-acid X → Adenylic acid + deaminised X

Embden and his co-workers regarded however the process as usually a reversible one, with a diminution of the free ammonia during the recovery period. Parnas and also Nachmansohn determined the isometric coefficient relating tension development and ammonia production, viz.:

$$\frac{\text{grams tension} \times \text{centimeter muscle length}}{\text{grams ammonia N.}}$$

and found it to be fairly constant under certain conditions. Nachmansohn (58) found, for tetani of 3 to 10 seconds (autumn frogs), that the isometric time coefficient was a very high one, 4500.10^6 for the NH_3/N compared to the lactic acid coefficient of 65.10^6. Considering the two in terms of molar transformations, 11 molecules of lactic acid would be produced for 1 of ammonia. The ammonia production must play a subordinate rôle in the energy transformations in muscle.

It was not until 1929 (Lohmann, 41; Fiske and Subbarow, 24) that it became evident that adenylic acid did not exist free in muscle but in the form of a compound in which there were two additional labile molecules of H_3PO_4. At first Lohmann (42), (43) regarded this so-called pyrophosphate fraction as being free in muscle, but later he and also Fiske and Subbarow prepared salts of adenosine-triphosphoric acid in which two of the phosphoric acid molecules were rapidly set free on hydrolysis, while the third was comparatively resistant to acid hydrolysis. The position of the substituent pyrophosphoric acid molecule, if it be in pyro form, has not yet been determined, but certain observations of Lohmann, referred to by Meyerhof in his recent book, are of interest in relation to the probable constitution of the body. The linkage is probably with the free hydroxyl groups in the ribose fraction.

In order to determine the amount of phosphorus in this pyro-nucleotide form, different procedures may be adopted. When the creatine-phosphate of the acid extract of fresh muscle has broken down, there are in the extract o-phosphate, the pyro compound, and Embden's mono-ester. The first mentioned can be determined in the usual way from the terminal value by the colorimetric method and, as the Embden ester is very resistant to acid hydrolysis, the increment in o-phosphate after 7 to 14 minutes' hydrolysis (in normal HCl at 100°) will give the pyro fraction value of the nucleotide and $1\frac{1}{2}$ times this value will give the pyro-nucleotide phosphorus. If the pyro-nucleotide be separated in the form of a calcium or silver salt after preliminary precipitation with acid Hg-acetate (Fiske and Subbarow) and its total phosphorus and 7 minutes' hydrolysis values estimated, the amount of the body and its freedom from admixture can be determined. The yield of adenyl pyrophosphate by the Fiske and Subbarow procedure may with care be a very good one.

Changes in the pyro fraction of the nucleotide under different conditions. Lohmann (42, 43) investigated these changes in the resting and in the stimulated amphibian muscle, and he was led to conclude that, under normal physiological conditions, the pyro content of intact muscle re-

mained constant. Even after moderate stimulation there was but little diminution to be observed, but after more prolonged stimulation it decreased, being accompanied by an increase in the o-phosphate. Re-synthesis of the ortho- to pyro-form has been observed by Embden and his co-workers in the aerobic recovery period, without any increase in the mono-ester (17). After strong stimulation an aerobic increase in the pyro fraction was not observed. They also refer to a peculiar early increase in the pyro fraction occurring in contracting weighted muscle over and above the value found in the non-weighted muscle. They regard therefore a synthesis of pyrophosphate as well as a breakdown in lactacidogen as early changes in a muscular contraction. After autolysis in $NaHCO_3$–KCl solutions at 40° the pyro fraction breaks down. The enzymic hydrolysis of the pyro fraction is checked in the presence of orthophosphate. Lehnartz (32) has shown that the enzymic synthesis of pyrophosphoric acid (really adenyl pyrophosphoric acid) is accelerated by the addition of adenylic acid to the alkaline solution. The recent work of Lundsgaard in Meyerhof's laboratory, dealing with monoiodo-acetate poisoning, indicates that the o-phosphate split off from the pyro fraction may be in large part accompanied by the production of hexose phosphates in mono- and di-ester forms.

A synthesis of the pyro-nucleotide can be readily effected in fresh muscle extracts containing adenylic acid and o-phosphate. The synthesis is accompanied by the setting free of basic valencies. Re-amination of any inosinic acid formed from the removal of the amino group in the adenylic acid is evidently essential before the synthesis of the pyro compound can take place. Following therefore the sequence of changes which occur when the pyro molecule breaks down, namely, the setting free of one molecule of ammonia and two of o-phosphate, accompanied by the replacement of the amino-purine by the oxy-purine, there must be a re-amination of the latter prior to the synthesis of the free o-phosphate and the nucleotide. At present very little is known regarding the relationship of the changes in the acid soluble phosphorus holding constituents to one another in the resting intact mammalian muscle, and in the same muscle aerobically active. It may be of some interest to refer briefly to the altered distribution produced by prolonged activity in rabbit's muscle frozen in situ, removed and analysed (unpublished analyses). (Table 1.)

During a subsequent recovery period it was found that the phosphagen was rapidly reformed, while the adenyl pyrophosphate still continued to break down and the mono-ester formation went on. The changes in

the pyro-nucleotide both in breakdown and recovery are less marked and take place more slowly than in the case of the phosphagen.

The rôle of the nucleotide in carbohydrate metabolism. Euler and Myrbäck and Meyerhof (18, 19, 20, 21, 49) regard it as part of the glycolytic and yeast ferment systems and closely associated with the co-ferment. Meyerhof has directed special attention to the effects produced by purification of the co-ferment solutions on the glycolytic process. If glycogen and phosphate be added to a solution containing purified lactic acid ferment and purified co-ferment there may be little or no glycolysis. Meyerhof and Lohmann recognised that the body required to activate the ferment system was adenyl pyrophosphate. The components in the co-ferment are evidently required for the early stage of labile ester formation and the setting free of the reactive sugar, and apparently not for the later stages. The mechanism of the action of this nucleotide, when the ferment co-ferment system reacts with hexoses, will repay further careful study.[1]

TABLE 1

	BICEPS FEMORIS (RESTING)		SAME (AFTER 7 MINUTES' TETANUS)	
	Milligrams	Per cent of total	Milligrams	Per cent of total
Total acid soluble, P. per gram muscle....	1.387		1.387	
Of this:				
As free ortho.....	0.265	19.1	0.798	57.5
Phosphagen.....	0.382	27.5	0.057	4.1
mono-ester.....	0.198	14.2	0.234	16.8
adenyl pyrophosphate.....	0.456	32.8	0.310	22.3
Recovered.....	1.301	93.6	1.399	100.7

3. GLYCOGEN. The cycle of changes concerned in the anaerobic breakdown of glycogen to lactic acid, and the oxybiotic recovery in muscle has been much more intensively studied than the earlier and more rapid pre-lactic acid changes concerned with the setting free of o-phosphate and ammonia. Disturbances in the sequence of the changes in the carbohydrate cycle are at the basis of all functional disturbances in muscle metabolism. Our present knowledge of the carbohydrate changes we owe especially to the investigations of Fletcher and Hopkins, and Meyerhof and Embden (25, 26, 27, 31, 49). As

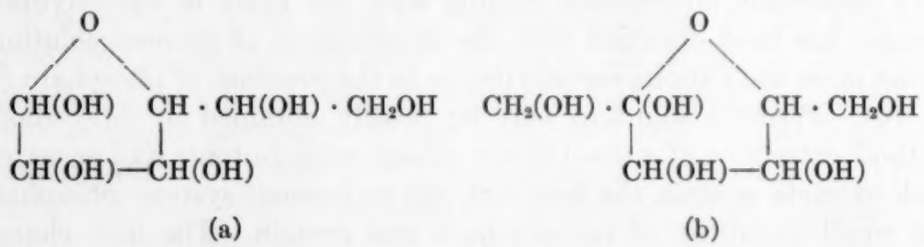
[1] Recently Lohmann (1931, Biochem. Zeitschr., ccxxxvii, 445) has shown that magnesium is an essential constituent of the co-ferment system.

numerous excellent accounts of this subject have appeared not only in monograph form (49) but are also now incorporated in all textbooks of physiology and biochemistry, it is quite unnecessary to give here what would merely be a brief résumé of carbohydrate metabolism in muscle. There are however certain points of physiological interest in connection with the storage of glycogen, the action of muscle ferments and the post-mortem changes in the carbohydrates of muscle to which attention may with advantage be directed. Reference will be made especially to subjects which require further investigation.

a. *The storage of muscle glycogen.* The glycogen store may be rapidly depleted by indirect or direct electrical stimulation of the muscle, and during the recovery period the carbohydrate may be comparatively rapidly replenished in the normal animal, while in the depancreatised animal the storage is checked (56). Best, Hoet and Marks (6) have shown that, in the resting eviscerated animal, injections of insulin increase the glycogen content of muscle. Recently Debois in Hoet's laboratory (8) has investigated the recovery process in the muscles of the cat under different experimental conditions. After decerebration and section of the cervical cord, or section of both vagi in the neck, or with intact vagi and removal of the pancreas, the animal loses the power of increasing the muscle glycogen during a recovery period. When only the right vagus was cut, storage occurred. In all cases where storage during a recovery period was prevented, the injection of 30 units of insulin gave rise to a renewed deposition. It is evident from Debois' and from earlier work that there is a hormonal control over the muscle glycogen storage.

b. *The muscle ferments and their action.* The cycle of changes in the breakdown of carbohydrate is effected by enzymes, the carbohydrate primarily acted upon being glycogen. It is only under special conditions that glucose added to ferment extracts breaks down to lactic acid. Very important information dealing with the cycle of carbohydrate changes has been obtained from the employment of ferment solutions acting upon the various carbohydrates in the presence of phosphate (4, 44, 70). Ferment solutions may be readily obtained by Meyerhof's method, extraction of minced frozen muscle with isotonic KCl or water. Such extracts contain the ferments, the co-ferment system, phosphate and small quantities of carbohydrate and protein. The first change which the glycogen undergoes is a diastatic one, brought about by the action of the muscle amylase, a ferment which is more thermostable than the others in the extract (44). In addition to hexoses found at this stage, there is a reducing saccharide of larger molecule which is apparently not a source of lactic acid. Fluoride does not interfere with

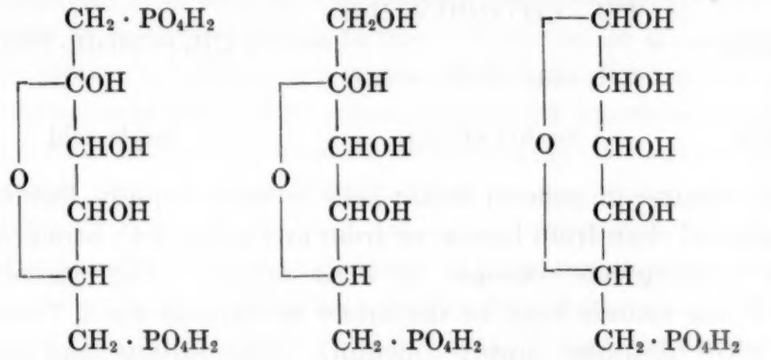
this action. The hexoses are then esterified in the presence of orthophosphates, the esters which are formed differing in the number and nature of their phosphoryl-linkages according to the conditions under which the ferments are acting. The process may be checked at this stage by the action of fluoride (Embden), and the ester formed in this case is a di-ester of a form more resistant to acid hydrolysis than the well known Harden-Young di-ester (Lohmann's stable ester 1, (38)). During the esterification process in the presence of fluoride there is a gradual rise in acidity of approximately the same order as when lactic acid production is proceeding in the absence of fluoride (4), the esters which are formed having higher dissociation constants than the corresponding ones of o-phosphoric acid (54). During any process in muscle therefore when hexose esters accumulate or break down, the disturbances in acid-base equilibrium must be borne in mind. During the normal carbohydrate cycle in muscle the esters which are formed must be much more labile than those which have been isolated. As intermediate products their amount in muscle will depend upon the relative rates of esterification and hydrolysis, and it is probable that the amounts actually in ester form in the absolutely resting normal muscle are small. Their formation and breakdown are intimately associated with the transformation of the hexose into a readily reactive form and it appears probable that the hypothesis of Meyerhof may give a true picture of the general outline of the change, one reactive molecule of a mono-ester breaking down to a reactive hexose molecule and a free phosphoric acid molecule, the latter reacting with another mono-ester molecule to form a more stable di-ester. The free γ-sugar of the reactive type does not appear to be capable of separate existence, but adopting the Haworth nomenclature (30) the γ-aldo-hexose (a) and γ-keto-hexose (b) may be shown with the following configurations (as furanoses).



(a) (b)

There may however be also the accumulation of a less reactive monoester which does not undergo the changes above referred to, seeing that the only ester which is present in fresh muscle, and which varies in

amount under different conditions is the Embden mono-ester. Lipmann and Lohmann (38) have recently studied the behaviour of various biological hexose esters to acid hydrolysis and have referred to the conditions under which hexoses with firm phosphoryl linkages are formed. Our knowledge regarding the position of the phosphoryl linkages in hexose esters is based mainly on the work of Morgan, Robison and coworkers (31a, 57, 66a) and Levene and Raymond (36, 36a). The three well known biological esters have probably the following structures:



(Harden-Young di-ester) (Neuberg mono-ester) (Robison mono-ester)

The Robison ester has been recently investigated carefully by King, McLaughlin and Morgan (31a), by Robison and King (66a) and by Levene and Raymond (36a). Until recently this ester, isolated from the products of yeast fermentation, has been by no means pure, but has contained a number of components, a glucose monophosphate, one or more fructose monophosphates, and probably also higher saccharide esters. The glucose monophosphate (Robison's ester), which constitutes 50 to 70 per cent of the hexose monophosphate, has probably the phosphoric acid group attached to the sixth carbon atom. Levene and Raymond from their comparison of the synthetic glucose-6-phosphate with the purified Robison ester are also now of the opinion that the latter is glucose-6-phosphate and not a glucose-5-phosphate with the unstable furanose ring, as they originally surmised. There are however certain differences between the synthetic ester and the natural aldose monophosphate, recently isolated by Robison and King, so that the subject requires further investigation.

The constitution of the Embden ester is still unknown, but in most respects it is closely allied to the Robison mono-ester. In the breakdown of the hexose after the hydrolysis of the ester, the intermediate 3 C.

atom fraction which appears to be the precursor of lactic acid is almost certainly methyl glyoxal (5). From the recent work of Neuberg and his co-workers, it should be possible to identify its presence in muscle extracts under different conditions. That hexose diphosphate does break down in part through a methyl glyoxal stage becomes evident in conditions when the glyoxalase activity is diminished (3). On the entrance of water (glyoxalase action) the methyl glyoxal is converted into lactic acid.

$$C_6H_{12}O_6 \longrightarrow \begin{matrix} CH_3 \cdot CO \cdot CHO + H_2O \\ CH_3 \cdot CO \cdot CHO + H_2O \end{matrix} \longrightarrow 2\ CH_3 \cdot CHOH \cdot COOH$$

hexose methyl glyoxal lactic acid

In animal tissues in general lactic acid is more rapidly formed from methyl glyoxal than from hexose or from any other 3 C. atom fraction.

c. *The carbohydrate changes in dead muscle.* The carbohydrate cycle in living muscle may be disturbed in various ways, from interference with hormone supply (insulin), from fatigue and from the action of poisons (fluoride, monoiodoacetate, etc.). Among these disturbances one must recognise the possibility of an interference with the colloidal condition of muscle so that the conditions under which the ferment system acts become abnormal. Embden and his co-workers have referred to this aspect of the subject. Deuticke and others (9) have shown that there are physico-chemical post-mortem changes in muscle which result in the prevention of the esterification to the di-ester stage, such as occurs under the action of fluoride on fresh muscle. The diminution in the solubility of the proteins has been taken as the measure of the colloidal change. Similar changes in protein solubility and esterification have also been observed in fatigued muscle, in rigor, etc. (9), (2). Macleod and his co-workers (1, 48, 68) have made a careful study of the carbohydrate changes in the intact mammalian muscles after death. In the first place glycogenolysis takes place very slowly so long as the muscles remain intact, even when they are kept at room temperature for an hour. There may occasionally be even a rise in the glycogen value in the post-mortem period. The lactic acid which always accumulates under these conditions bears no definite relationship to the amount of glycogen which has disappeared. There is also no definite relationship between the free sugar and the lactic acid production. On the other hand Macleod and Simpson found that when frozen muscle powder was thawed, glycogen disappeared much more rapidly than the lactic acid

accumulated. Attention therefore requires in both cases to be paid to the nature of the intermediate products from which the lactic acid arises in the former, and into which the glycogen is transformed in the latter case. It is just in this intermediate stage of the carbohydrate cycle that disturbances may readily occur. The inherent difficulties in the determination of the nature and amounts of these intermediate reducing substances make it at present impossible to come to any decision regarding the nature of the disturbed cycle. Macleod and his co-workers have directed attention mainly to the hexoses, higher saccharides or hexose phosphates as possible intermediaries. It would be of interest to get a clearer picture of alterations in the phosphate distribution in intact dying muscle, especially when one considers the recent work of Lundsgaard on muscle passing into an action rigor. Disturbances in the carbohydrate cycle in yeast fermentation are so readily brought about by changes in the medium that probably similar changes take place within the muscle. It is possible that an interference with the colloidal mechanisms of muscle gives rise to the disturbed carbohydrate cycle which is evident in the form of the "alactacid" contractions met with in monoiodoacetate poisoning.

4. Lactacidogen. We owe the original discovery of a hexosephosphoric acid ester in muscle to Embden, who at the beginning regarded its constitution as similar to that of the well known Harden-Young hexose-diphosphate of yeast fermentation. In 1927 Embden and Zimmermann (15) definitely established its constitution as that of a hexose monophosphate. Embden and his co-workers early recognised that after prolonged stimulation and also after the autolysis of minced muscle in alkaline solution there was an increase in o-phosphate as well as of lactic acid, which they believed to be derived from a breakdown of lactacidogen. Recently it has been shown that the increase in o-phosphate under these conditions is mainly due to the setting free of labile phosphoric acid molecules from creatine-phosphoric and adenyl-pyrophosphoric acids. Embden has however obtained an increase in o-phosphate along with an equivalent disappearance in the ester when a muscle is killed at the height of a contraction evoked by immersion in liquid air. At the moment of relaxation a resynthesis of the ester takes place. A part of the hexose breaks down to form lactic acid, and fresh hexose is obtained by glycogenolysis to replace the amount which has disappeared. The interpretation given by Embden of the changes which occur on the immersion of fresh intact muscle in liquid air has been questioned by Eggleton and Lohmann. It is necessary therefore to refer briefly to

his experimental procedure. When a fresh unstimulated muscle is immersed in liquid air it contracts and is fixed in the contracted state. The other muscle, which is taken to be the control one, is in the first place fatigued by a short tetanus and then when relaxed it is fixed in the relaxed state by immersion in the liquid air. The former is regarded by Embden as the quickly fixed *contracting* muscle, the latter as the relaxed *resting* muscle. In the former the amount of orthophosphate (total inorganic) is greater than in the latter while the amount of ester in the latter is greater than in the former. The increase in inorganic phosphate in the former is equivalent to the diminution in the ester value. The Eggletons have obtained similar results but they regard, as does Lohmann also, the former muscle as the resting one and the latter as a muscle which has undergone a change due to fatigue. Lohmann recently (45) has repeated Embden's procedure and has compared the inorganic and ester phosphorus values of fresh muscles fixed in liquid air with those obtained for similar muscles fixed in trichloroacetic acid. As he finds these values unaltered by the method of fixation he concludes that there is no significant breakdown of lactacidogen on immersion in liquid air, and the reason for the lower inorganic phosphate and higher ester values in the muscle killed in the relaxed state after tetanic stimulation is the synthesis of the ester in the post-excitation period. It is apparently impossible to fix a muscle at the height of a single contraction and so the chemical changes at this moment must remain uncertain. It appears certain however that the amount of the mono-ester does go on increasing for some time after the stimulation of the muscle has ceased. Embden's view is that in a momentary contraction the three chemical changes which occur are all associated with the setting free of basic valencies, viz., phosphagen breakdown, splitting of the hexose-phosphoryl linkage in lactacidogen, and deamination of the adenyl-pyrophosphoric acid. We shall now pass to the subject of "alactacid" contractions.

THE CONTRACTION PROCESS WITHOUT LACTIC ACID PRODUCTION. Regarding the sequence of the various chemical changes which occur in active muscle and their importance in the energy cycle, there have naturally been differences of opinion. Certain changes, such as phosphagen breakdown, the deamination of the nucleotide, and, according to Embden, the breakdown of lactacidogen, have been associated with the earliest stages in muscular contraction, while others such as lactic acid formation and still later esterification and hydrolysis of the pyro fraction of the nucleotide have been regarded as the accompaniments of

subsequent stages, when the known anaerobic chemical changes in muscle are considered from the energy standpoint. At present it appears as if only two of these, phosphagen breakdown and lactic acid production, can be regarded as playing important parts. It must also be remembered that the chemical processes associated with the energy balance must not only take place rapidly, but must also be capable of a rapidly reversible reaction. A disturbance in the metabolism in muscle may be due to an interference with the recuperative phase in a normally reversible action. The recent work of Lundsgaard (46, 47) has opened up an important field for investigation and an endeavour will be made to give a concise account of the results which he has obtained and to discuss their significance. After the injection into the frog's dorsal lymph sac of a neutralised solution of monoiodoacetic acid (0.4 mgm. per gram) a condition of progressive rigor sets in which may remain for some hours before death. The sequence of the muscular changes which has been observed (in this laboratory) in frogs poisoned with monoiodoacetate may be briefly summarised. After injection of 0.4 mgm. per gram body weight and at a room temperature of 15° to 19° spasmodic movements are first seen after 15 to 30 minutes. Sometimes these take the form of violent strychnine-like convulsions with hyperextension, at other times of fibrillar contractions of the muscles. At this period stimulation of the toes produces greatly exaggerated reactions, leaving the hind limbs strongly contracted and drawn up. After 20 to 30 minutes the muscles of the shoulder girdles and the fore limbs show fibrillar twitchings and these continue, somewhat irregularly, until the fore limbs become stiff and pass into rigor usually after 40 to 60 minutes. Five to ten minutes after the contractions of the fore limb muscles, similar movements appear in the thorax and spread backwards. From half to one hour after the injection the twitchings involve the upper muscles of the hind limbs, quickly spreading downwards until the feet begin to twitch. The movements start feebly, increase in strength and then, becoming feeble again, finally cease. In 80 to 120 minutes a state of complete rigor is established. Sometimes after a preliminary convulsion involving the whole body, the process starts in the muscles of the neck and upper limbs and then travels backwards to the hind limbs. Lundsgaard observed that the establishment of the rigor evidently depends upon an active state of the muscles, because narcotisation or curarisation or denervation prevents the onset. Thus section of the nerves of a limb prevents the rigor spreading to the paralysed muscles, and subsequent stimulation of the motor nerve evokes a short series of

contractions culminating in a state of maximal rigor. The reaction of the rigid muscles, using a simple colorimetric method, was found by Lundsgaard not to have shown the usual swing to the acid side. When the lactic acid production was estimated in normal muscles and in poisoned muscles which were kept in a thermostat at 40° for two hours, in the former there was the usual well marked increase while in the latter the value remained practically the same as at the outset. Sometimes the amount of lactic acid was even lower at the end than at the outset. The same disturbance was evident in the rabbit's muscles after monoiodoacetate injection. Reaction determinations (electrometric) which have been made in this laboratory illustrate clearly the difference in behaviour of the control and the poisoned muscles of the frog.

	pH AT OUTSET	pH AFTER 40 HOURS AT ROOM TEMPERATURE
Normal	7.21	5.92
Poisoned		7.03–7.10

Lundsgaard found on direct stimulation in situ of the poisoned frog's gastrocnemius that 110 to 150 single isotonic contractions were carried out without lactic acid production, while the normal gastrocnemius stimulated in the same way produced 0.5 to 0.6 mgm. lactic acid per gram muscle. In the poisoned muscle the phosphagen was completely broken down, while in the normal controls the breakdown was only partial. The phosphoric acid which was set free did not however appear in the inorganic form but there was a large increase in hexosephosphate. Calculating the ester phosphate as the Embden mono-ester (using the Embden-Jost reduction method) the phosphoric acid set free could not be entirely accounted for by the increase in hexosephosphate. The energy production of the stimulated poisoned muscle could be approximately calculated from the phosphagen breakdown and its hydrolysis heat value compared with the energy equivalent of the lactic acid produced in the control muscles under similar working conditions. From Lundsgaard's earlier observations it appeared that the hydrolysis heat value of the phosphagen breakdown agreed remarkably closely with the heat equivalent of the lactic acid production in the controls. In a later paper (47), issuing from Meyerhof's institute, Lundsgaard has given the isometric coefficients of the phosphagen breakdown in the poisoned muscles under anaerobic conditions. As has already been

mentioned Nachmansohn found that the isometric coefficient $\left(\frac{\text{centimeter length of muscle} \times \text{gram tension}}{\text{gram } H_3PO_4 \text{ set free}}\right)$ of the normal frog's muscle was not constant throughout a contraction period but increased with the duration of the excitation from 89.10^6 to 375.10^6. In the poisoned muscle on the other hand Lundsgaard found that throughout a wide range of tension development a remarkable constancy in the isometric coefficient for single stimuli, and also for the isometric time coefficient for tetani (1 to 4, each of 2 secs. duration), i.e., $\frac{\text{L (cm.)} \times \text{T (gram)} \times \text{seconds}}{\text{gram } H_3PO_4}$, the average of the former being 51.10^6, and the latter $15.2.10^6$. In curarised normal muscle Nachmansohn found that the isometric coefficient for single contractions rose owing to the diminished phosphagen breakdown compared to the tension development. In muscles obtained from frogs curarised while under the action of monoiodoacetate, Lundsgaard found that the two isometric coefficients were approximately the same as in the case of non-curarised frogs under monoiodoacetate, being respectively 50.10^6 and $13.1.10^6$ (average values). It is evident therefore that the phosphagen breakdown in the poisoned muscles is not affected by curarisation. The parallel course of the tension development and the phosphagen splitting is most evident when overstimulation of the muscle is prevented as in the case of curarised muscle. As Nachmansohn had drawn attention to the possible relationship between chronaxie values and phosphagen breakdown, Lundsgaard determined the former in the poisoned muscles and in the curarised poisoned muscles. He found that the chronaxie values of normal and monoiodoacetate muscles were the same and that, after curarisation of the latter, the chronaxie values rose in the same way as in normal curarised muscle. As the poisoned muscles during anaerobic activity showed, unlike the normal muscles, almost a complete breakdown of phosphagen, a resynthesis of the phosphagen anaerobically appeared improbable. The partial anaerobic synthesis of phosphagen in normal muscles is the accompaniment of a lactic acid production, from which the energy might be derived for the endothermic synthesis, but in the poisoned muscle there is no reaction known which could serve this purpose. Even if in the normal muscle lactic acid production does not occur sufficiently rapidly in order that it should be made use of for the rapid anaerobic synthesis of phosphagen, it is extremely probable that there are rapidly occurring intermediate reactions in the normal carbohydrate cycle which might be available. When a poisoned muscle is examined immediately

after a tétanic period, and another, stimulated in the same circuit, is examined after a period of anaerobic rest ($\frac{1}{2}$ to 2 minutes), the phosphagen in the latter is found to be lower than in the former so that if the breakdown be incomplete in the working period it continues during the post-excitation period. An anaerobic resynthesis of phosphagen was evidently impossible.

The oxidative resynthesis of phosphagen in the poisoned muscles. The rate of phosphagen breakdown is much slower when the resting poisoned muscles are kept in oxygen instead of nitrogen. On stimulation of poisoned sartorii kept in Ringer's solution, saturated with oxygen and nitrogen respectively, much more work can be performed under the aerobic conditions. The muscle, working under anaerobic conditions, passes into rigor at a time when the other with the free oxygen supply is still performing as much work as at the outset. In the former the phosphagen is found to have broken down almost completely while in the latter only about one-third of the original store may have been used up.

The chemical changes in the poisoned muscles. In the first place the hexose, derived from the glycogenolysis, both in the resting muscle in the anaerobic state and in the muscle which has undergone fatigue by stimulation and then has passed into rigor, is apparently practically completely esterified so that the inorganic phosphate shows no increase. The ester which is mainly formed is a di-ester which behaves similarly on acid hydrolysis to the Harden-Young hexose diphosphate, but a mono-ester, resistant to acid hydrolysis and conforming in reducing power to the Embden ester, is also produced. The esterification process is a somewhat delayed one, and is only extremely slight when the muscle is frozen during a contraction, while after a two minutes relaxation period the process is almost complete. The onset of rigor is usually associated with complete esterification. As regards the changes in the adenyl-pyrophosphate more work requires to be done. Apparently the pyro fraction in the resting poisoned muscle is not altered in amount, but after stimulation it breaks down, two molecules of phosphoric acid and one of ammonia being set free. After prolonged stimulation the chemical changes which the muscle has undergone are the following: almost complete breakdown of phosphagen and the pyro fraction of the nucleotide, along with the esterification of the hexose and the phosphoric acid produced by the enzymic hydrolysis, and ammonia production from deamination of the nucleotide. The essential feature of the disturbance in the muscle metabolism is apparently the transformation of the normal reversible processes in the carbohydrate cycle into irreversible

ones. The Lundsgaard hypothesis is that in the normal muscle contraction, phosphagen constitutes the direct energy producing substance and the lactic acid production is essential for the synthesis of the creatine and phosphoric acid set free. It is impossible with our present knowledge to give any satisfactory explanation of the part played by lactate in phosphagen synthesis. It is absolutely essential however to bear in mind the other disturbances in the chemical processes, namely, the deamination of the nucleotide and probably the disturbance in re-amination, the setting free of the labile phosphoric acid molecules of the adenyl pyrophosphate, and the diversion of the hexose into more or less stable ester forms with all the phosphoric acid set free from the phosphagen and the pyro-nucleotide. Regarding the interest and the importance of these investigations dealing with "alactacid" contractions there can be no doubt, but one must await further investigation before a clear picture of this abnormal carbohydrate cycle can be presented.

If this be true in the case of this abnormal type of muscle metabolism, when the processes concerned all tend to become irreversible, the difficulties become still greater when an endeavour is made to transfer information so obtained to the intricate cycle of carbohydrate transformation met with when a normal muscle is excited. The anaerobic synthesis of the phosphagen in normal muscle and its absence in the poisoned muscle, affecting as they do the earliest synthetic change in the cycle, are of primary importance. Lundsgaard expresses the opinion that in curarised normal muscles the check in phosphagen breakdown may be due to the fact that the resynthesis of the phosphagen is facilitated, Meyerhof and Nachmansohn having found that in curarised muscle the percentage resynthesis of broken down phosphagen is greater than in normal muscle. When an anaerobic synthesis is impossible as in the poisoned muscle, then, as has been found, curarisation does not check the breakdown. The investigations of Lundsgaard must naturally lead to a careful reconsideration of the earlier work on muscular metabolism and its relationship to the contraction process, especially as regards the reaction factor in the shortening phase (see Bethe, 7).

THE REACTION CHANGES IN INTACT MUSCLE. The correlation of reaction determinations made in injured muscle in the resting and fatigued states with the chemical processes which disturb acid base equilibrium in intact muscle is extremely difficult (66), and hence the importance to be attached to new methods of attacking this problem. Among the most fruitful recent lines of research in this domain, one must include the investigation of reaction changes using the Warburg manometric

technique. Lipmann and Meyerhof (39) have made use of this method for the study of the reaction changes in intact resting and contracting normal muscles of the frog, and also in the muscles poisoned by monoiodoacetate. The method employed by Lipmann and Meyerhof for the study of the reaction changes in the anaerobic resting state and during activity depends upon the manometric measurement of the carbonic acid absorbed or set free from the muscle in Ringer solutions containing $NaHCO_3$ (0.02 to 0.03 mol.), phosphate (0.03 per cent P), and carbonic acid. The reaction of the solution was varied by the passing of carbonic acid of different concentrations through the solution. In this way a range of reaction from pH 7.23 (solution equilibrated with 5 per cent CO_2) to pH 6 (equilibrated with 100 per cent CO_2) could be obtained. If acid equivalents arise or basic ones disappear, carbonic acid will be set free and the amount can be manometrically determined using the Warburg technique. For stronger acids or bases passing into the carbonic acid-bicarbonate solution, there will be an equivalent amount of carbonic acid given off or absorbed respectively. The effect produced by the internal buffer salts of the muscle can be experimentally determined and the necessary corrections made by the employment of a CO_2 retention factor. For the higher CO_2 pressures and 0.02 to 0.03 molar $NaHCO_3$ the factor is only slightly over 1 (1.06 to 1.10).

As far as the phosphagen is concerned the electro-titration curves enable one to determine conveniently at each fixed reaction the basic equivalent of the phosphagen breakdown which can react with the carbonic acid (fig. 1). The chemical processes which are associated with the setting free of acidic valencies are lactic acid production, the establishment of hexose phosphoryl linkages in esterification, and the hydrolysis of the pyro fraction of the adenyl-pyrophosphoric acid, while those accompanied by the setting free of basic valencies are the hydrolysis of the creatine-phosphate and the deamination of the nucleotide. Prior to the employment of this method, but little could be said regarding the early changes in reaction accompanying a short period of contraction. The earlier work mainly refers to the relationship between the lactic acid produced within the fatigued muscle and the resultant disturbance in the acid-base equilibrium (28), (66). It has been recognised that even under those conditions although the predominant change is due to the lactic acid production, there are other changes such as those already referred to, which contribute to the alteration in reaction. In short periods of muscular contraction it is to be expected that chemical processes other than lactic acid production should have a signifi-

cant effect on reaction. Thus, as Nachmansohn has shown, in an extremely short isometric tetanus (2 seconds) approximately four molecules of phosphagen break down for one of lactic acid produced. At the normal reaction of fresh intact muscle, probably the phosphagen breakdown produces only a slight disturbance in reaction, but such as it does must be in the direction of a slight swing to the alkaline side. From a study of the electrotitration curves of I, creatine-phosphoric acid, and II, the products of its hydrolysis (see fig. 1), it is evident that within the reaction zone of physiological importance (pH 6 to 8) basic valencies are set free during the splitting process—approximately 0.1 NaOH equivalent at pH 8 rising to a maximum of 0.8 base equivalent at pH 6.

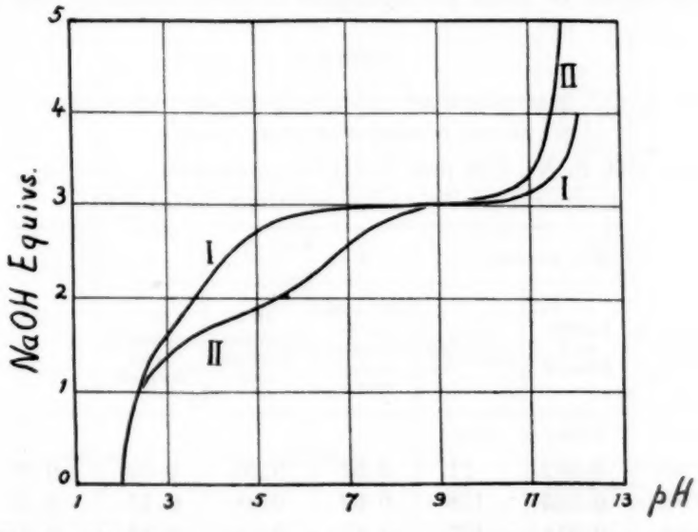


Fig. 1. Electro-titration curves of creatine-phosphoric acid and its components, with equivalents of NaOH. *I.* Creatine-phosphoric acid. *II.* Its components. (Lipmann and Meyerhof.)

The undecomposed phosphagen has little or no buffering action at the physiological reaction of resting muscle, but after hydrolysis the curve exhibits the normal buffering influence of the orthophosphate in this zone.

Meyerhof and Lipmann employed the method in the first place for the investigation of the changes in the *intact normal resting muscle under anaerobic conditions* when kept in the bicarbonate solution equilibrated with carbonic acid-nitrogen mixtures, with CO_2 varying between 5 per cent and 100 per cent. When the muscle was kept in Ringer's solution, with a 5 per cent CO_2 in nitrogen mixture filling the gas space in the vessel and at a temperature of 20° (reaction pH 7.23), the pressure re-

mained practically steady for three hours, but with higher carbonic acid concentrations (lower pH) basic valencies were set free as shown by CO_2 absorption. The absorption increased with a rise in the CO_2 concentration. Up to 4 or $4\frac{1}{2}$ hours the muscle exposed to 100 per cent CO_2 (pH 6) showed an increase in alkalinity. After this period carbonic acid was given off due to processes associated with acid production (lactic acid, etc.). In order to determine whether the alkalisation could be accounted for from the amount of phosphagen broken down minus the lactic acid produced, it was necessary to determine the P_2O_5 set free and the lactic acid which had been formed. From the P_2O_5 in milligrams set free per gram muscle, the base equivalents can be determined by multiplying the number of mols phosphagen broken down by the equivalent

TABLE 2

Determination of CO_2 absorption, phosphagen breakdown and lactic acid production in the resting anaerobic muscle

(In 38 per cent CO_2 in N_2, 0.02 mol $NaHCO_3$, phosphate—pH approximately 6.4, temperature 20° and equivalent factor 0.64)

DURATION OF EXPERIMENT	PER GRAM MUSCLE			a	b	c	b − c	10^{-5} EQUIVALENT CO_2 ABSORBED
	P_2O_5-phosphagen set free	Lactic acid formed	CO_2 absorbed	10^{-5} mol phosphagen broken down	10^{-5} equivalent base set free	10^{-5} equivalent acid formed		
hours	*mgm.*	*mgm.*	*cmm.*					
2	0.40	0.082	91	0.57	0.36	0.09	0.27	0.39
$3\frac{1}{2}$	0.70	0.135	158	0.99	0.64	0.15	0.49	0.71
5	0.83	0.311	137	1.17	0.75	0.34	0.41	0.63

base factor for the particular reaction. This value can then be compared with the base equivalent of the mols carbonic acid bound as determined from the carbonic acid absorption. With the higher carbonic acid concentrations, lactic acid production is so slight that it may be disregarded when compared with the predominant phosphagen breakdown. A table of results given by Meyerhof and Lipmann illustrates clearly the method which they employed (table 2). An examination of the figures in the last two columns shows that the CO_2 equivalents absorbed are greater than can be accounted for by the excess basic valencies set free by the phosphagen breakdown. There will be a certain increment in basic valencies over and above those derived from phosphagen breakdown from the deamination and the setting free of ammonia from the nucleo-

tide. If the explanation which has been given of the reaction changes in the anaerobic resting muscle be correct, then *during an aerobic recovery period* there ought to be a reversal of the changes. That is to say during the restitution period, when there is a synthesis of the products of the phosphagen breakdown, the setting free of carbonic acid should take the place of the absorption observed in the anaerobic condition. Accompanying the phosphagen synthesis in the recovery period, there will be also a removal of lactic acid with the setting free of base which will diminish the amount of CO_2 driven off by the increase in the acid

TABLE 3

a. The anaerobic breakdown

DURATION	CO_2 IN N_2	MOL $NaHCO_3$	pH	PER GRAM MUSCLE	
				Equivalent base neutralised by CO_2 absorbed $\times 10^{-5}$	Equivalent base from phosphagen breakdown $\times 10^{-5}$
hours	*per cent*				
3	100	0.030	6.15	0.86	1.1
$3\frac{1}{4}$	62.5	0.020	6.15	0.49	0.6

b. The aerobic synthesis

DURATION	CO_2 IN O_2	MOL $NaHCO_3$	pH	PER GRAM MUSCLE	
				Equivalent acid from CO_2 set free $\times 10^{-5}$	Equivalent acid from phosphagen synthesis $\times 10^{-5}$
hours	*per cent*				
$1\frac{1}{4}$	80	0.03	6.25	0.36	0.69
1	62.5	0.02	6.15	0.16	0.23

valencies which is the accompaniment of the phosphagen synthesis. The respiratory CO_2 may be taken as balanced by the O_2 removal. The condensed table 3 from Lipmann and Meyerhof will illustrate the essential nature of the reaction changes in the two processes, the anaerobic breakdown and the aerobic synthesis of phosphagen under the usual experimental conditions. The disparity between the values in the last two columns during the recovery period is due in part to the increase in base from the disappearance of lactic acid diminishing the amount of CO_2 given off.

THE REACTION CHANGES DURING ANAEROBIC STIMULATION. The re-

lationship between the absorption and setting free of CO_2 at various reactions of the medium and the accompanying chemical changes, has been very carefully investigated by the two authors. During the stimulation of muscle the accompanying chemical processes which will disturb reaction are the phosphagen breakdown, the lactic acid production and to a lesser extent the ammonia production. Hexose ester formation and the hydrolysis of the pyro fraction of the nucleotide will be insignificant if the stimulation be not excessive. The most interesting results

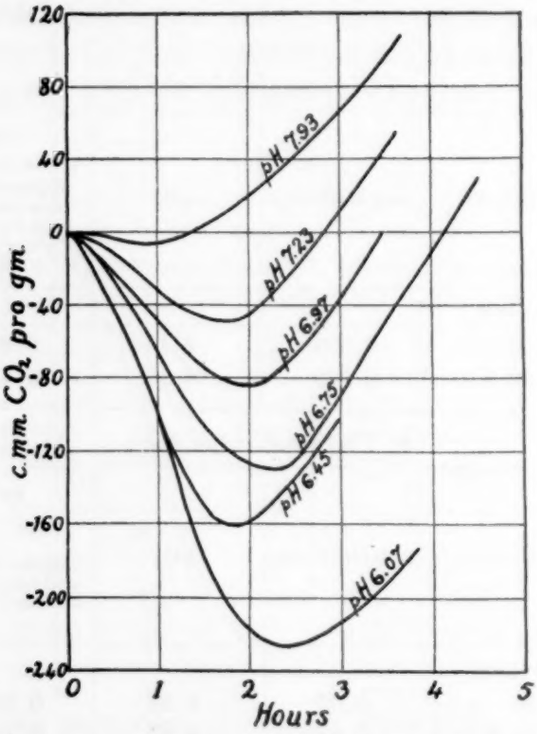


Fig. 2. Reaction changes in anaerobically stimulated normal muscle. $-CO_2$ values = absorption of CO_2. (Lipmann and Meyerhof.)

were obtained from slow stimulation, three single stimuli per minute, when the chemical changes were investigated as well as the manometric readings. Figure 2 illustrates the changes in the carbonic acid over a period of five hours with different reactions of the medium. At the most alkaline reaction (pH 7.93) the manometric reading shows a very slight CO_2 absorption for the first hour and then acid production becomes evident as shown by the setting free of CO_2. As the reaction of the medium is made more acid, alkalisation becomes more and more prominent, succeeded at later intervals by excess acid production. After a

certain period of activity the manometric reading comes back to the original one, when the CO_2 absorbed during the earlier stimulation is balanced by the CO_2 set free during the later. At this time the muscle should contain the same amount of bound carbonic acid as at the outset and the excess base set free in the earlier period should be neutralised by the excess acid produced in the later one. An analysis of the muscle at this time should show equivalence between the lactic acid formed and the combined base derived from the phosphagen splitting and the deamination of the nucleotide. The figures given by the authors for the two experiments show a close correspondence between the acid and base equivalents as chemically determined at the time when the manometric readings indicate that acid-base equilibrium has been again established. As the acid and base equivalents of three processes alone have been determined, it is evident that no other chemical change can have a distinct

TABLE 4

GAS MIXTURE, CO_2 IN N_2	pH	1 EQUIVALENT ACID (LACTIC) $\times 10^{-5}$	MOL phosphagen BROKEN DOWN $\times 10^{-5}$	EQUIVALENT FACTOR	2a EQUIVALENT BASE FORMED FROM PHOSPHAGEN $\times 10^{-5}$	2b EQUIVALENT BASE (AMMONIA) $\times 10^{-5}$
per cent						
15	6.8	0.59	1.44	0.36	0.50	0.06
34.5	6.5	0.69	0.93	0.60	0.56	0.07

influence on the reaction. In table 4 the acid equivalents produced are shown in column 1, and the base equivalents produced in columns 2a and 2b.

THE REACTION CHANGES IN MUSCLES UNDER THE INFLUENCE OF MONOIODOACETATE. The chemical changes in the muscles in this condition have been already referred to. The predominant change in reaction must be due to the phosphagen breakdown, as there is but little or no lactic acid produced. There are however the other chemical changes which contribute to any such reaction shift, hexose esterification, ammonia production and the hydrolysis of the pyro fraction of the nucleotide. Lipmann and Meyerhof have followed by the manometric method the carbonic acid absorption in the resting II and active I poisoned muscles (fig. 3). The manometer vessels were filled with a 34.5 per cent CO_2 in nitrogen gas mixture and the readings taken at short intervals. The active muscle was stimulated four times per minute for three quarters of an hour. Alkalisation was evident both in the case of the resting

and the active muscle, occurring much more rapidly in the latter, but in both cases the final values for the carbonic acid absorption were approximately the same. The figure shows that in the later stage there is no process resulting in excess acid formation either in the resting or active muscle. When the poisoned muscle is stimulated in a bicarbonate-CO_2 solution of approximately neutral reaction, there is practically no change in reaction on stimulation because the basic equivalents set free by the breakdown of the phosphagen and the production of ammonia are balanced by the acid equivalents set free by esterification and by hydrolysis of the pyro fraction. At more acid reactions (e.g., pH 6.4) when CO_2 absorption takes place during the excitation of the poisoned

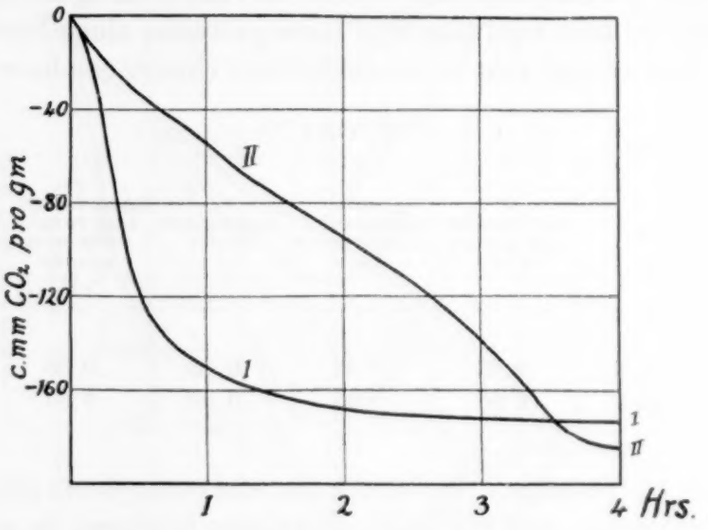


Fig. 3. Poisoned muscle. I. Stimulated for 45 minutes (4 stim. per sec.) pH 6.5. II. Resting muscle. (Lipmann and Meyerhof.)

muscle, calculation, from the chemical analyses of the base equivalents set free by the phosphagen breakdown and the acid ones set free by hexose esterification and the hydrolysis of the pyro-nucleotide, shows an extremely close correspondence between the base equivalents of the CO_2 absorbed and the excess base equivalents over the acid ones in the chemical processes referred to. This agreement of the manometric with the chemical determinations is strikingly shown in a table given in Lipmann and Meyerhof's paper, the section of which dealing with the calculation of base and acid equivalents may be studied with advantage. It is perhaps advisable in the first place to describe very briefly the principle of the method by which these figures have

been obtained. The amounts of free phosphate, phosphagen and pyrophosphate P_2O_5 per gram muscle were determined in the resting anaerobic muscle and in the muscle anaerobically stimulated in the free gas space (carbonic acid nitrogen gas mixture). The diminution in the sum of the three phosphate constituents in the stimulated muscle compared to the sum of the same in the resting muscle gives the amount of P_2O_5 which has been esterified (as hexose ester). The amounts of P_2O_5 derived from the phosphagen and the pyrophosphate breakdown and the amount used up for esterification during the active period are then calculated as mol fractions of phosphagen and adenyl pyrophosphate broken down and of phosphate esterified. On multiplying these mol fractions by their respective equivalent factors the base or acid equivalents set free in each case are determined. The equivalent factor for

TABLE 5

Changes produced by anaerobic activity of poisoned muscle (monoiodacetate)

AT pH	PHOSPHAGEN			ADENYL PYROPHOSPHORIC ACID			ESTER FORMATION			EXCESS BASE EQUIVALENT $\times 10^{-5}$	BASE EQUIVALENT OF CO_2 ABSORPTION $\times 10^{-5}$
	Mol broken down $\times 10^{-5}$	Base equivalent factor	Equivalent base $\times 10^{-5}$	Mol broken down $\times 10^{-5}$	Equivalent factor (including NH_3)	Equivalent base formed $\times 10^{-5}$	Mol phosphate esterified $\times 10^{-5}$	Acid equivalent factor	Equivalent acid formed $\times 10^{-5}$		
6.4	1.86	0.61	1.14	0.31	0.5	0.15	0.72	0.25	0.18	1.11	1.3
7.15	1.83	0.25	0.48	0.45	0	0	2.70	0.25	0.67	−0.19	0.09

the breakdown of the adenyl-pyrophosphoric acid, based upon Lohmann's unpublished titration curves, comprises two factors, one for the ammonia production which is calculated as a full base equivalent and the other (acid equivalent) for the setting free of two mols of o-phosphoric acid. The latter is taken as 0.5 acid equivalent at pH 6.4 and a full acid equivalent at pH 7.15 so that at the former reaction the equivalent factor is 0.5 (as a base equivalent) and at the latter the acid and base equivalents being equal cancel out. The excess base equivalents set free during a period of anaerobic activity in the poisoned muscle are calculated in this way and can then be compared with the base equivalents of the carbonic acid absorption (manometrically determined) after the necessary correction has been made for retention of carbonic acid by the buffering substances of the muscle, which varies from 5 per cent of the absorbed CO_2 in the case of a 60 per cent carbonic acid mixture to 25 per cent with 4 per cent CO_2. The resting muscle was kept for

1½ hours in the manometer until absorption of CO_2 had ceased, the other, after 20 seconds' tetanus leading to complete exhaustion, remained in the vessel until absorption of CO_2 had also ceased (1 to 1½ hours after stimulation). The muscles were then examined for the constituents referred to. The calculated values for the three chemical changes, as observed in two of the experiments, may be given in illustration of what has been already discussed (table 5).

OTHER "ALACTACID" CONTRACTIONS. 1. *Fluoride poisoning.* As is well known fluoride even in low concentrations, checks lactic acid production in minced muscle and in muscle extracts. Lipmann (40) has recently investigated the effects produced by fluoride injections in frogs and has obtained results somewhat similar to those described by Lundsgaard in monoiodoacetate poisoning. The fibrillar contractions and the onset of rigor were observed also in the fluoride muscles, and the rigor was prevented in a limb by section of the nervous supply. Both in the resting and in the active muscle the changes are of the same kind, namely, very slight lactic acid production, breakdown of phosphagen and pyrophosphate accompanied by well marked esterification. Lipmann refers to certain differences between the two forms of poisoning but these need scarcely be referred to because the metabolism of fluoride poisoned muscle in the active state requires further investigation, especially with regard to the recovery processes, before its essential nature can be established.

2. *After exhaustion of the muscle glycogen.* Can a muscle contract after its glycogen store has been completely exhausted? It is difficult to answer this question because it is practically impossible to free a muscle completely from glycogen. It is in addition extremely difficult to determine the nature and the amount of other carbohydrate forms present, which are capable of producing lactic acid. The study of the action of insulin on the muscle glycogen in normal animals brought up this question again. Olmsted and Harvey (62) came to the conclusion that the muscles of frogs could be rendered practically free from glycogen (less than 0.2 mgm. per gram muscle) by insulin convulsions, and that in this condition they were still capable of contracting for hours upon being stimulated by maximal induction shocks, the height of the contractions being practically the same as in the normal muscles. In a later paper Olmsted and Coulthard (61) gave the lactic acid as well as the glycogen values in the stimulated muscles of insulinised frogs. They found that in frogs, suffering from insulin convulsions, the glycogen store was very small, and after stimulation resulting in fatigue

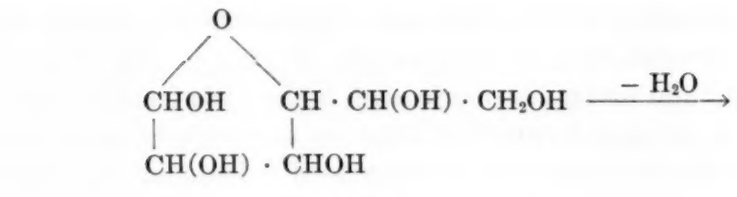
and subsequently rigor, there was only a slight further loss in glycogen and a slight increase in lactic acid, the gain in the latter being greater than the loss in the former. Such muscles in rigor mortis contained however only a small quantity of lactic acid (0.05 per cent). The lactic acid coefficient of work done was not determined. These experiments were carried out at a time when no attention had been directed to the other energy producing process, namely, the breakdown of creatine-phosphoric acid.

Recently the question has again been considered by Ochoa in Meyerhof's institute (60) and although, owing to certain difficulties in determining the small amounts of carbohydrate in the muscles at the end of a stimulation period, the results are not so definite as one would desire, still certain conclusions can be drawn with some degree of certainty. The glycogen of the muscles of frogs was brought down to a low level by insulin convulsions, and the work done under aerobic conditions along with the oxygen consumption was determined. The oxidation quotient showed that the energy requirements for the work done could certainly not be covered by the oxidation of carbohydrate. In the case of the muscles acting under anaerobic conditions although the lactic acid produced could have been obtained from the carbohydrate present, the isometric coefficient of the lactic acid was much too high, and the lower the original carbohydrate content of the muscle, the higher did the quotient rise. It was evident that in all cases where this quotient (K_{mL}) rose above 300.10^6, the tension development could not be covered by the energy equivalent of the lactic acid formed. In such cases the phosphagen breakdown was relatively great, but the isometric coefficient of the phosphagen breakdown showed that the necessary energy could not have been furnished even by this process. The material and energy changes which take place in muscles carrying out work under conditions when the carbohydrate stores are very low require further investigation.

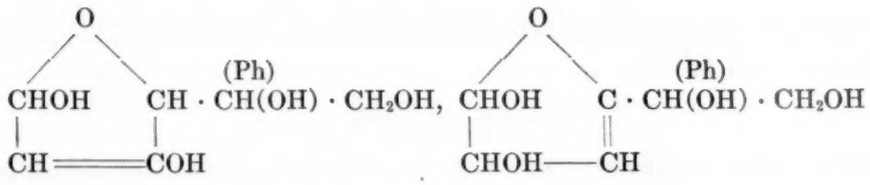
THE SEQUENCE OF THE CHEMICAL PROCESSES IN A NORMAL CONTRACTION. It is impossible at present to give the sequence of the chemical processes initiated by the excitation of muscle and the part played by each in the energy changes of the contraction process.[2] It is evident that the chemical processes concerned in the contraction process itself are entirely anaerobic ones, the energy set free necessitating reactions of great velocity and such as are reversible in nature. The breakdown also must affect a much larger number of molecules than if the process

[2] Embden's views regarding the sequence of the changes in muscular contraction are given under lactacidogen.

were one of oxidation, while the restitution synthetic processes only require the oxidative removal of a fraction of the products of anaerobic breakdown or the equivalent of this fraction, say, in the form of hexose. The relationship between the two chemical processes which play the predominant part in muscle contraction, namely, phosphagen breakdown and lactic acid production, has not yet been made clear but the sequence of the changes may at least be suggested. The earliest change is probably associated with the setting free of phosphoric acid from its linkage with creatine. This is followed by the entrance of the substituent acid groups into the hexose derived from the enzymic hydrolysis of glycogen. The normal esterification process probably leads to the formation of labile mono-ester forms, and from these by enzymic hydrolysis H_3PO_4 is set free along with the reactive sugar or rather the products of the breakdown of the reactive sugar in the form of 3 C. atom fractions, probably methyl glyoxal, which is then transformed into the stable end product, lactic acid. The anaerobic synthesis of creatine-phosphoric acid from some of the H_3PO_4 set free is an essential stage in the normal contraction process. The absence of such anaerobic synthesis, as in "alactacid" contractions, is accompanied by the diversion of the phosphoric acid into more stable mono- and di-ester forms in which the hexose fraction is resistant to the normal breakdown into the 3 C. atom fractions. The adenyl pyrophosphoric acid undergoes deamination probably early in the contraction phase, and this may be accompanied by the setting free of the loosely fixed phosphoric acid molecules. The breakdown of the pyro fraction evidently continues for some time after a prolonged contraction period and, if the normal glycolytic process be interfered with, resynthesis of the orthophosphate and adenylic acid is evidently checked. The relationship of the adenyl pyrophosphate changes to those more directly associated with the anaerobic breakdown of carbohydrate requires further investigation. As regards the changes in the hexose fraction which take place in order that the molecule may readily undergo breakdown, only those who are engaged in the study of the constitution of the carbohydrates are competent to express an opinion. The suggestion by Bernhauer and Tschinkel (5), based upon the study of the carbohydrate breakdown by hydrogen peroxide, that the biological degradation of sugar may take place through the stage of a labile methylglyoxal-aldol with a phosphoric acid linkage (forms b or c) appears to be worthy of consideration. Assuming the furan ring structure for the body, its relationship to γ-aldo-hexose (a) is clear



(a)



(b) or (c)

As Fiske and Meyerhof have pointed out the orthophosphate set free by the phosphagen or other breakdown at the normal reaction of the muscle must increase the buffering capacity of the tissue. If along with the production of one molecule of lactic acid two or three molecules of phosphoric acid are set free at pH 7 sufficient base will be obtainable to neutralise almost the whole of the lactic acid produced without any alteration in the hydrion concentration of the muscle. The changes in reaction during the contraction and relaxation processes in muscle as a whole are so slight as to make it extremely difficult to establish any causal relationship between hydrion concentration and the mechanisms concerned in these processes. During the oxidative restitution in amphibian muscle the following chemical changes are to be observed:

1. The completion of the synthesis of the products of phosphagen breakdown.

2. With a sufficient oxygen supply the entire removal of the lactic acid which was formed in the contraction process and the resynthesis of glycogen associated with the increased oxygen intake so that the amount of glycogen in the muscle after recovery is the same as that at the outset minus the lactic acid equivalent which has undergone oxidation. The chemical processes associated with the oxidative resynthesis of lactic acid are still unknown. Apart from lactic acid pyruvic acid seems to be the most probable intermediary, stimulating as it does the respiratory activity of the recovering muscle, and also being capable of increasing the glycogen content of amphibian muscle. The energetics of the possible transformations of pyruvic acid to hexose have been discussed by Meyerhof (49).

3. The re-amination of the deaminated nucleotide at the expense of the oxidative deamination of amino-acids (Parnas). The resynthesis of adenylic acid and orthophosphoric acid, if the pyro fraction has been broken off in a prolonged period of activity, is evidently under normal conditions a delayed process and in the case of "alactacid" contractions is checked.

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